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FORMATION, ADSORPTION, AND DEGRADATION OF *N*-NITROSOATRAZINE IN WATER AND SOIL

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FORMATION, ADSORPTION, AND DEGRADATION OF *N*-NITROSOATRAZINE IN WATER AND SOIL

by

Hsin-Ro Wei

A THESIS

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Master of Science

Major: Natural Resource Sciences

Under the Supervision of Professor Patrick J. Shea

Lincoln, Nebraska

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FORMATION, ADSORPTION, AND DEGRADATION OF *N*-NITROSOATRAZINE IN WATER AND SOIL

Hsin-Ro Wei, M.S.

University of Nebraska, 2011

Advisor: Patrick J. Shea

The products of xenobiotic reactions in environmental mammalian systems may pose risks equal to or greater than parent compounds. Products of concern include nitrosamines, which can be carcinogenic, mutagenic, and teratogenic. Nitrosamines may form in soil, lake water, and sewage; they also can form in agricultural soils treated with agrichemicals containing amine moieties and nitrogen fertilizer. The nitrosamine-forming reaction of amines with nitrite is promoted at acidic pH. The widely used herbicide atrazine has secondary amine groups that can react with nitrite to form *N*-nitrosoatrazine (NNAT). The primary objective of this research was to characterize the formation, stability, and adsorption of NNAT in water and soil. NNAT formed most readily in acidic solution (pH 2-4) and in soil at pH $\leq$ 5. Acetate and fulvic acid promoted NNAT formation in water at pH 4 to 7. NNAT was relatively stable in solution in a two-month experiment. However, under light, NNAT rapidly

degraded in solution, and atrazine concentration increased, indicating hydrolysis (denitrosation) of NNAT to atrazine. In soil containing atrazine and nitrite, NNAT formed after 7 d at pH 4 and after 14 d at pH 5, but no NNAT was found at pH 6 and 7. No NNAT was detected in pH 4 soil under oversaturated or anaerobic conditions, indicating the importance of oxygen in the nitrosation reaction. Adsorption K_d and K_{oc} values show greater adsorption of NNAT (average $K_d = 5.93$ and $K_{oc} = 495$) than atrazine (average $K_d = 2.71$ and $K_{oc} = 123$) in Aksarben silty clay loam at agronomic soil pH. Adsorption coefficients decreased in the order: NNAT in Aksarben > NNAT in Rosebud silt loam > atrazine in Aksarben > atrazine in Rosebud > NNAT in Valentine sand > atrazine in Valentine soil. Larger desorption K_d values indicate greater hysteresis of NNAT than atrazine. NNAT half-life in Aksarben soil was approximately 9 d, with degradation to atrazine and other compounds. This information is important when evaluating atrazine fate and impacts in soil-water environments.

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INTRODUCTION

The transformation products of xenobiotics in environmental matrices and mammalian systems may pose risks equal to or greater than the parent compounds. Reaction products of concern include nitrosamines, which can be carcinogenic, mutagenic, and teratogenic and may affect nitric oxide (NO) levels in cells, critical in signaling growth and biological functioning (Turjanski et al., 2000; Lai and Chou, 2008). Low concentrations of nitrosamines have been found in food, beverages, sunscreens, osmetics, pesticide products, cigarette smoke, and automotive exhaust fumes (Mirvish, 1975; Williams, 2004). Nitrosamines also may be generated in humans from the reaction of nitrite with amines (Gatehouse and Tweats, 1982; Cova et al., 1996; Rostkowska et al., 1998).

Previous research indicates that nitrosamines may form in soil, lake water, and sewage, affecting ecosystems (Ayanaba et al., 1973a; Mirvish, 1975; Tate and Alexander, 1975; Kearney et al., 1977; Pancholy, 1977; Oliver and Kontson, 1978; Trevisan et al., 1998). Nitrosamines can form in agricultural soils treated with pesticides and veterinary pharmaceuticals containing secondary amine moieties and receiving heavy applications of nitrogen fertilizer (Ayanaba et al., 1973a; Mirvish, 1975; Khan and Young, 1977; Kearney et al., 1977; Oliver and Kontson, 1978; Khan,

1981; Zwickenpflug, 1994). The reaction is promoted at acidic pH (Mirvish, 1975; Khan, 1981; Mallik et al., 1981).

Atrazine is widely used in agriculture with approximately 35 million kilograms annually world used. It is nitrosatable, and relatively little is known about the formation, fate, and availability of *N*-nitrosoatrazine (NNAT) in soil-water systems. Other agricultural chemicals such as simazine, ziram, propoxur, and benthiazuran have been shown to be nitrosated readily (Eisenbrand et al., 1975). Tate and Alexander (1975) reported that nitrosamines can be stable in soils and sewage, and leaching to groundwater is a concern (Dean-Raymond et al., 1977). To our knowledge, there is little published research on the adsorption and availability of nitrosamines in soil. In addition, the literature suggests that some solutes may promote nitrosamine formation in soil and water (Cova, et al., 1986; Weerasooriya and Dissanyake, 1989), but information in this area is limited.

The objectives of this research are to characterize the formation, stability, and adsorption of NNAT and the nitrosamine products from the reaction of atrazine and other agrichemicals with nitrite in water and soil.

LITERATURE REVIEW

N-Nitrosamine Formation

Nitrosation of organic compounds generally requires conversion of nitrite to nitrous acid (pka = 2.8; Riordan et al., 2005), which explains why nitrosation is catalyzed by acid. Under these conditions the HNO_2 is converted to the nitrous acidium ion ($H_2NO_2^+$), which subsequently forms the nitrosonium ion (NO^+), a strong nitrosating agent (Figure 1; Mirvish, 1975).

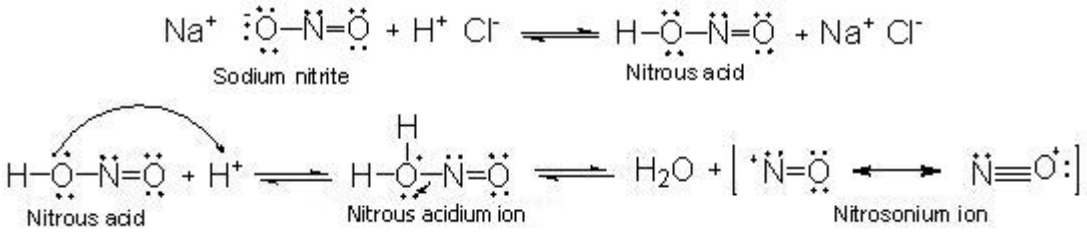


Figure 1. Conversion of nitrite to the nitrosonium ion.

In aqueous solution, HNO_2 is in equilibration with dinitrogen trioxide (N_2O_3), which is also a highly effective nitrosating agent (Eq. 1; Williams, 2004):

$$HNO_2 \rightleftharpoons N_2O_3 + H_2O \quad (1)$$

The presence of non-basic nucleophiles (X^-) can result in the formation of a third nitrosating species, XNO (Eq. 2; Williams 2004):

$$HNO_2 + H_3O^+ + X^- \rightleftharpoons XNO + 2H_2O \quad (2)$$

To our knowledge, pure nitrous acid has never been isolated, because decomposition

occurs giving various oxides of nitrogen as final products. This decomposition is usually represented by Eq. (3) (Williams, 2004)

$$3HNO_2 = 2NO + HNO_3 + H_2O \quad (3)$$

$$2NO_2 + 2H_2O \rightleftharpoons HNO_2 + NO_3^- + H_3O^+ \quad (4)$$

$$2HNO_2 \rightleftharpoons NO + NO_2 + H_2O \quad (5)$$

$$2NO + O_2 \rightarrow 2NO_2 \quad (6)$$

In the absence of oxygen, the decomposition pathway involves the two equilibria, Eq. (3) and (4), whereas in the presence of oxygen Eq. 6 also occurs and decomposition is significantly faster. The decomposition may cause slower nitrosation reactions.

Secondary amines react with nitrite via the nitrosonium ion (NO^+) under acidic conditions to form nitrosamine derivatives (Eq. 7; Mirvish et al., 1991):

$$R_1NHR_2 + NO^+ \rightleftharpoons R_1NH^+NOR_2 \quad (7)$$

$$R_1NH + NOR_2 + H_2O \rightarrow R_1NNOR_2 + H_3O^+ \quad (8)$$

Nitrosamines may be produced from the reaction of nitrite with agrichemicals or their breakdown products containing amine moieties. Nitrite is generated from ammonium or nitrate fertilizers via nitrification and denitrification. The nitrification is from the biological oxidation of ammonia with oxygen into nitrite. Denitrification occurs where oxygen, an electron acceptor, is depleted, and bacteria respire as a substitute terminal electron acceptor. It only takes place in anaerobic environments

where oxygen consumption exceeds the oxygen supply and where sufficient quantities of nitrate are present. These environments may include certain soils and groundwater, wetlands, oil reservoirs, poorly ventilated corners of the ocean, and in seafloor sediments. The herbicide atrazine is a weak base (pka = 1.68; Weed Science Society of America, 2007) with two secondary amine groups and is nitrosatable (Figure 1). In previous research NNAT was not detected in soil at pH 2.5 to 5.3 (Kearney et al., 1977).

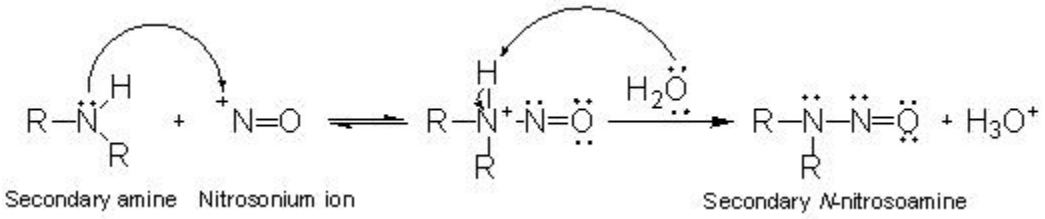


Figure 2. Formation of *N*-nitrosoatrazine from atrazine and nitrosonium ion.

Atrazine (1-chloro-3-ethylamino-5-isopropylamino-2, 4, 6-triazine; IUPAC name) has been widely used in the U.S. and world-wide. In 2002, atrazine use for agricultural purposes was 35 million kg of active ingredient per year (Gianessi and Reigner, 2006). It is a selective pre- and post- emergent herbicide used to control broadleaf and grassy weeds. In the Midwestern states atrazine has been widely used as a corn herbicide (USEPA, 1994). In the early 1990s, approximately 68% of the corn, 50% of the sweet corn, 65% of the sorghum, and 94% of the U.S. sugarcane acreage was treated with atrazine (Gianessi and Anderson, 1995). Atrazine is also used for selective weed control in conifers, primarily Christmas trees and ornamentals, and on

ecofallow land periodically left untilled for soil and moisture conservation (Weed Science Society of America, 2007).

The fungicide thiram (tetramethylthiuram disulfide) can be decomposed by bacteria and algae to form tertiary amines (Ayanaba et al., 1973b). The tertiary amines may then be degraded to secondary amines which react with nitrite to form nitrosamines in soil. Tate and Alexander (1974) found dimethylamine (DMA) and diethylamine can be produced in soil from decomposition of dimethyldithiocarbamate and diethyldithiocarbamate, respectively.

Janzowski (1980) reported that nitrosation of pesticides in aqueous suspensions is strongly influenced by pH. Wolfe et al. (1976) formed NNAT from reaction of 0.1 mM atrazine with 25 mM nitrite in water at pH 1 to 5 and the reaction rate increased with the square of the nitrite concentration between 10 and 100 mM. NNAT was reported to have a short half-life in water (17 and 114 h at pH 2 and 3, respectively), but was very stable at pH greater than 4 (Wolfe et al., 1976). Kearney et al. (1977) showed that chemical conversion of atrazine to NNAT increased as soil pH decreased, but NNAT was unstable in water (pH 2.5~4.5) and soil (pH 2.5-5.3 and pH 7.7), and degraded to atrazine and polar products. Despite this earlier work, a better understanding of nitrosamine fate is needed to properly assess risk and promote management practices that minimize risks to humans and the environment. Cova et al.

(1986) showed the acetate greatly enhances the rate of *N*-nitrosation of curzate (1-(2-cyano-2-methoxyiminoacetyl)-3-ethylurea). The *N*-nitrosation of curzate appears maximal at pH 2.5, reaching a 10 % conversion when acetate was present. Weerasooriya et al. (1989) catalyzed nitrosodibutyl amine (NDBA) formation with fulvic acid. The optimum pH for NDBA formation was 3.5; the concentration of NDBA in samples containing fulvic acid was about twice that of samples without fulvic acid. Thus the presence of certain solutes can affect nitrosation kinetics.

Adsorption of *N*-Nitrosamines in Soil

NNAT was slightly less mobile than atrazine in four soils tested by Kearney et al., (1977). Soil pH could not account for this difference. Gan et al. (2006) reported rapid leaching of *N*-nitrosodimethylamine (NDMA) in irrigated soil, suggesting low adsorption; however, companion studies showed that much of the NDMA was lost via gaseous diffusion and volatilization in unsaturated soils (Arienzo et al., 2006). Kearney et al. (1977) mentioned that soil pH could not account differences in NNAT mobility in soil, but we could not locate other information on the adsorption and desorption of NNAT.

Degradation of *N*-Nitrosamines in Solution and Soil

NDMA is decomposed by UV light to produce its precursors DMA, methylamine, nitrite, and nitrate (Lee et al., 2005a, 2005b). NDMA was quite stable in lake water, where no degradation or loss was observed during 3.5 months of monitoring (Tate and Alexander, 1975). Slow disappearance was noted in soil after a lag of several weeks. The loss was rapid in sewage, but even after two weeks more than half the added nitrosamine remained (Tate and Alexander, 1975). Gan et al. (2006) observed NDMA was relatively persistent in turfgrass soils. Yang et al. (2005) reported greater persistence of NDMA in soil during cooler seasons, when organic matter is low, and microbial activity is limited. Young et al. (1977) found that NNAT was relatively stable in solution and soil, although Wolfe et al. (1976) reported rapid decomposition by light and acidic conditions in water to atrazine and DEA. Kearney et al. (1977) found denitrosation was one of the primary mechanisms of NNAT transformation, since atrazine was the major product identified in the soil extracts. However, Tate and Alexander (1975) and Oliver et al. (1979) reported that biodegradation was primarily responsible for nitrosamine degradation in soil.

MATERIALS AND METHODS

Herbicides, Soils, and Reagents

Herbicides. Herbicide names, formula, structure, molecular weight, and properties are reported in Table 1. Herbicides included: atrazine, cyanazine, simazine, ametryn, diuron, linuron, and tebuthiuron (purchased from Chem. Service, West Chester, PA) and hydroxyatrazine, deethylatrazine, and deisopropylatrazine (purchased from Sigma-Aldrich, St. Louis, MO).

Atrazine (ATZ) is widely used to control broadleaf weeds and some annual grasses in corn, sorghum, sugarcane, fallow, certain nuts, conifers and non-crop land. Atrazine has been detected in ground and surface waters (e.g., Spalding et al., 2003). Hydroxyatrazine (HA; 2-(ethylamino)-6-(propan-2-ylamino)-1H-1, 3, 5-triazin-4-one) is a major degradation product of atrazine in surface soils and acidic solution. Deethylatrazine (DEA; 6-chloro-2-*N*-propan-2-yl-1, 3, 5-triazine-2, 4-diamine) is a major product of microbial degradation of atrazine in soil. Deisopropylatrazine. (DIA; 6-chloro-*N*-ethyl-1, 3, 5-triazine-2, 4-diamine,) is also a product of atrazine biodegradation in soil.

Simazine (6-chloro-2-*N*, 4-*N*-diethyl-1, 3, 5-triazine-2, 4-diamine) is widely used to control broadleaf weeds and annual grasses in corn, berries, and in fruit and

nut orchards. Ametryn (4-*N*-ethyl-6-methylsulfanyl-2-*N*-propan-2-yl-1, 3, 5-triazine-2, 4-diamine) is used to control broadleaf weeds and annual grasses in corn, sugarcane, pineapple and other tropical crops, and in non-crop land. Diuron (3-(3, 4-dichlorophenyl)-1, 1-dimethylurea,) is used to control annual and perennial weeds in established alfalfa, corn, sorghum, and various fruit and nut crops, and agricultural crops. Linuron. (3-(3, 4-dichlorophenyl)-1-methoxy-1- methylurea) is used to control annual and perennial broadleaf and grassy weeds in corn, soybeans, sorghum, and various vegetable crops. Tebuthiuron (1-(5-tert-butyl-1, 3, 4-thiadiazol-2-yl)-1, 3-dimethylurea)) is used to control certain broadleaf weeds and woody brush in pasture, rangeland, and on industrial sites. The herbicides and degradation products mentioned above were available in the inventory of the Xenobiotics Laboratory, University of Nebraska-Lincoln. They can also be obtained from ChemService (West Chester, PA) or from the herbicide product manufacturers.

NNAT was prepared following the procedure of Brambilla et al. (1985). Atrazine (21.5 mg) and $NaNO_2$ (27.6 mg) were added to in 2 mL deionized, distilled (DD) water and the solution pH was adjusted to approximately 3.5 with 1M HCl. The mixture was shaken for 1 h in a 37^{o}C water bath in the dark. Then the solution pH was neutralized by adding $NaHCO_3$ and brought to a final volume of 3 mL with DD water. The reaction products were extracted three times with CH_2Cl_2 (3 × 10 mL), dried with

Na_2SO_4, and evaporated to dryness. The residues were dissolved in acetonitrile and purified by HPLC using a reverse phase C18 column (5µm, 250 × 4.6 mm) on an instrument equipped with a photodiode array detector and fraction collector. Conditions were as follows: λ = 240 nm; mobile phase, 50: 50 water/ acetonitrile; flow, 1.0 mL/min; injection volume, 100 µL. The purified aqueous NNAT was concentrated by rotary evaporator. Recovery at NNAT was approximately 89 %.

Soils. Soils (Table 2) and bentonite clay used in the experiments were available in the inventory of the Xenobiotics Laboratory. Bentonite clay was treated with dodecyltrimethylammonium bromide (DDTMA*;* purchased from Sigma-Aldrich (St. Louis, MO*)* to simulate lipid-coated clays that may be present in sediments, whereas the clay treated with tannic acid (TAC; purchased from Sigma-Aldrich, St. Louis, MO*)* was used to simulate clays coated with polar organic functional groups. The DDTMA-clay was prepared following the procedure of Sithole and Guy (1985). DDTMA was a hydrophobic molecule with a bromide. The bromide could interact with ion exchange sites to stick in the particle surface for simulate the lipid on clay. Briefly, an aliquot of the Na-clay suspension was equilibrated with 1 M DDTMA bromide for 15 h. The coagulated clay was filtered through Whatman No. 4 filter paper and washed with distilled water. The DDTMA-clay was dried overnight at 100 ^{o}C, ground in a micro-mill, and sieved through a 250 mesh size filter. The TAC-clay

complex was prepared following the procedure of Sithole and Guy (1985). Briefly, 1 M tannic acid was equilibrated with an aliquot of the Na-clay suspension for 15 h. The normally white clay turned a dark grey color after the reaction. The excess acid was removed by dialysis: the TAC-clay suspension was placed in 50 mL dialysis bags and dialysed six times overnight in 2 L of distilled.

Reagents and Organic Solutes. Sodium nitrite ($NaNO_2$), ammonium nitrate (NH_4NO_3), and humic acid were obtained from Sigma-Aldrich (St. Louis, MO). Sodium hydroxide (NaOH), hydrochloric acid (HCl), and acetate solution were obtained from Fish Scientific (Fair Lawn, NJ). Organic matter extract was prepared by mixing 10 mL deionized, distilled (DD) water with 1 mg Histosol soil (98% organic matter, obtained from North Carolina, U.S.A) in (water), and filtering to remove the solids. Humic acid solution was prepared following the same procedure used to prepare the organic matter extract. Fulvic acid was prepared following the procedure of Black (1965). In this procedure 40 g soil was placed in a polyethylene centrifuge bottle and 200 mL of 0.5 M NaOH solution was added. The mixture was shaken for 12 h and centrifuged. An additional 200 mL of 0.5 M NaOH solution was added to the soil, shaken for 1 h, and the centrifuging and decanting were repeated. The residue was dispersed in 200 mL DD water, centrifuged, and the supernatant added to the previous extracts. The pH of the resulting solution was adjusted to 1.0

with concentrated HCl and the humic acid was allowed to settle. The excess supernatant (fulvic acid) was removed from the acidified extract. The fulvic acid was dried, and 1 mg was dissolved in 10 mL DD water.

Formation of *N*-Nitrosoatrazine

Formation in Solution. A method for determining the potential for nitrosation in aqueous solution was initially described as the "nitrosation assay procedure" (NAP test; World Health Organization, 1978). For the NAP test used in this study, the secondary amine of each compound was reacted in aqueous (DD water) solution with sodium nitrite at a 1:4 molar ratio at room temperature and pH 2 to 7. This was done by adding 10 mL of 0.4 mM sodium nitrite and 10 mL of a 0.1 mM solution of each test compound in a Teflon tube and adjusting pH from 2 to 7 with 1.0 M HCl and 0.1 M NaOH. Samples were removed for HPLC analysis to monitor parent reaction product levels during two weeks of incubation at room temperature under aerobic and anaerobic conditions. The same procedure was repeated (under aerobic conditions) using simazine, cyanazine, ametryn, diuron, linuron, or tebuthiuron in place of atrazine. In separate experiments, acetate, humic acid, and fulvic acid, and organic matter extracts were added (100 μL of 100 mg/L solution) to evaluate their impacts on nitrosation of atrazine.

Formation in a Soil-Water Slurry and Soil. Soil slurries were prepared by adding 7.5 mL DD water to 2 g air-dried Aksarben soil in Teflon tubes. The pH of the suspension was adjusted from 2 to 6 with 1.0 M HCl and 0.1 M NaOH. Sodium nitrite (5.26 mg) and 0.02 mg of atrazine (nitrite: atrazine molar ratio of 156:1) were added to each tube, and the tubes were incubated for 14 d.

For soil experiments, Aksarben soil was prepared by adding 10 mL DD water to 10 g air-dried soil and allowing it to drain by gravity (to approach the field capacity). After 24 h, the soil contained 7.5 mL water. The pH of the soil was adjusted to 4, 5, 6, and 7 with 1.0 M HCl and 0.1 M NaOH. Sodium nitrite (5.26 mg) and 0.02 mg of atrazine (nitrite:atrazine molar ratio of 156:1) in 7.5 mL DD water were added and the tubes were incubated for 3, 7, 14, and 28 d, following the general procedures of Kearney et al. (1977) and Khan et al. (1977). The soil was extracted for 1 h twice with 10 mL of acetonitrile, extracts were centrifuged at 5000 rpm for 15 min, and 1 mL of supernatant was taken for HPLC analysis. This experiment was repeated in oversaturated soil under aerobic conditions and in an anaerobic chamber (Coy Laboratory Products Inc., Grass Lake, MI) to determine the influence of soil oxygen on nitrosation. “Oversaturated” soil was prepared by adding 15 mL water to 10 g of air-dried soil in a Teflon tube. For anaerobic chamber experiments, the water was degassed with nitrogen.

In additional tests, ammonium nitrate (28.6 mg) (fertilizer) and 0.02 mg of atrazine were added in 7.5 mL distilled water to 10 g air-dried Aksarben soil in a Teflon tube, incubated for 3, 7, 14, and 28 d (Kearney et al., 1977), then centrifuged and analyzed as previously described. Similar experiments were conducted in slurries of 10 g soil in 50 mL water. The pH was adjusted to 2, 3, 4, and 5 with 1.0 M HCl and 0.1 M NaOH.

Adsorption of *N*-Nitrosoatrazine in Soil

Soil adsorption of NNAT was determined by adding 2 g air-dried Aksarben soil to Teflon tubes with 10 mL DD water and 12 μg NNAT (6 mg/kg Aksarben soil). The pH was adjusted to 3-8 with 1M HCl and 1M NaOH, and the tubes were shaken for 24 h at 25°C. The suspensions were centrifuged and concentrations of NNAT in the supernatant were determined by HPLC. Desorption was determined by decanting the remaining supernatant, adding 10 mL DD water to the soil, shaking for 24 h at 25°C, and determining the concentrations of the compounds by HPLC. The experiment was repeated with atrazine for comparison with NNAT.

Adsorption and desorption distribution coefficients (K_d) and organic carbon partition coefficient (K_{oc}) were calculated as follows (Eq. 9, 10):

$$K_d = q / C \quad (9)$$

$$K_{oc} = K_d / f_{oc} \quad (10)$$

where q is atrazine or NNAT adsorbed (μmol/kilogram soil), C is micromoles of atrazine or NNAT per liter of supernatant after equilibration, and f_{oc} is the organic carbon fraction of the soil. Additional NNAT and atrazine adsorption experiments were conducted using Rosebud and Valentine soils, as well as bentonite clay, clay treated with DDTMA, and clay treated with TAC.

Degradation of *N*-Nitrosoatrazine in Solution and Soil

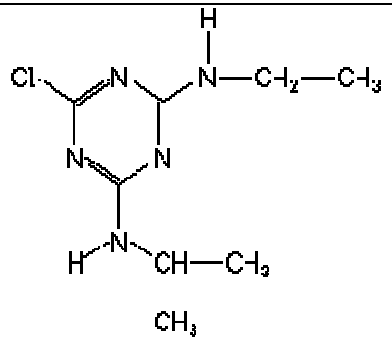
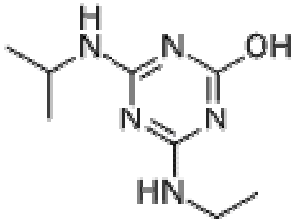
Degradation in Solution. Ten mL of 45 μM NNAT solution was placed in Teflon tubes and incubated under dark (tubes wrapped in foil) and light (laboratory/natural daylight) conditions. The selected solutes (acetate, humic acid, fulvic acid, or crude organic matter extract) were those used in the formation experiments. For testing the degradation rate may be affected by presence of the selected solutes, we added (100 mg/L final concentration) to determine their influence on the stability of NNAT. The solution pH was adjusted to 4, 6, and 8. The solution was subsampled periodically and analyzed by HPLC over a two week period.

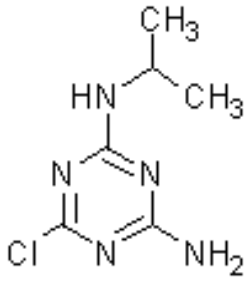
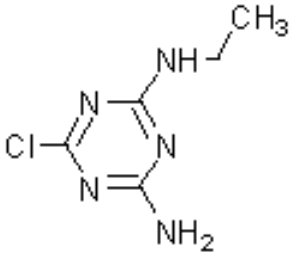
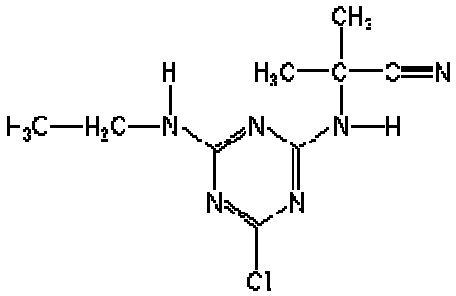
Degradation in Soil. Ten g air-dried Aksarben soil was placed in Teflon tubes containing 6.5 mL DD water and 50 µg parent compound or nitrosated product. Samples were incubated for 3, 5, 7, 9, and 14 d under aerobic conditions at 25 °C. Extractions were performed by shaking equal amounts of moist soil with 20 mL acetonitrile for 1 h twice. The extracts were centrifuged at 5000 rpm for 15 min and analyzed by HPLC. This experiment was repeated, following the same procedure, after adjusting soil pH to 4, 6, and 7.

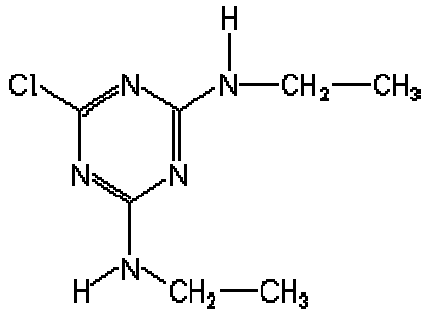
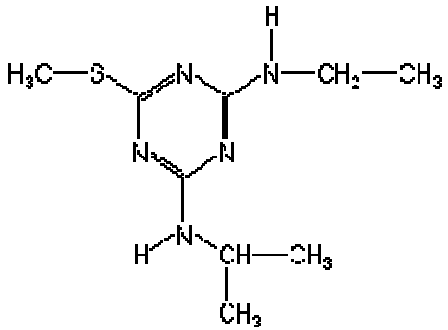
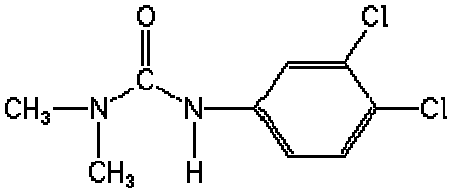
Chemical Analysis

Parent compounds and nitrosated products were quantified using HPLC (Shimadzu Scientific Instruments, Inc.-USA, Columbia, MD) by comparison with standards; operating conditions are given in Table 3. Sample chromatograms are given in the Appendix. LC-MS was used as required for confirmation and quantitative analyses.

Table 1. Agrichemicals included in the study

compound	formula	structure	Properties
atrazine	$C_8H_{14}ClN_5$		White solid molecular weight: 215.7 water solubility: 33 mg/L K_{ow} = 481 pka = 1.7
hydroxyatrazine (HA)	$C_8H_{15}N_5O$		White solid molecular weight: 197.2376 water solubility: very high K_{ow} = 158 pka = 5.2

deethylatrazine (DEA)	$C_6H_{10}ClN_5$		White solid molecular weight: 187.6 water solubility: very high K_{ow} = 158 pka = 1.4
deisopropylatrazine (DIA)	$C_5H_8ClN_5$		White solid molecular weight: 173.6 water solubility: 650 mg/L K_{ow} = 100 pka = 1.5
cyanazine	$C_9H_{13}ClN_6$		White solid molecular weight: 240.7 water solubility: 171 mg/L K_{ow} = 127 pka = 1.6

simazine	$C_7H_{12}ClN_5$		White solid molecular weight: 201.7 water solubility: 2 mg/L K_{ow} = 122 pka = 1.62
ametryn	$C_9H_{17}N_5S$		White solid molecular weight: 227.3 water solubility: 200 mg/L K_{ow} = 427 pka = 4.1
diuron	$C_9H_{10}Cl_2N_2O$		White solid molecular weight: 233.1 water solubility: 42 mg/L K_{ow} = 589 pka = none (non-ionizable)

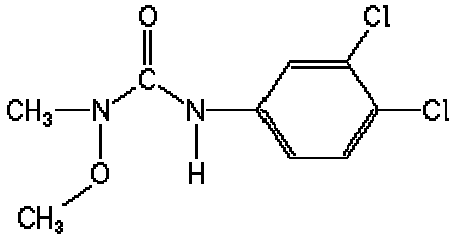
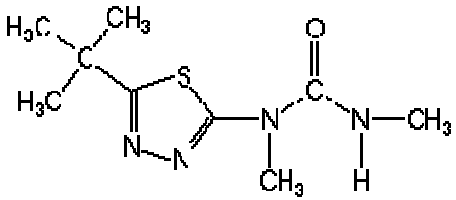
linuron	$C_9H_{10}Cl_2N_2O_2$		White solid molecular weight: 249.1 water solubility: 75 mg/L K_{ow} = 1010 pka = none (non-ionizable)
tebuthiuron	$C_9H_{16}N_4OS$		White solid molecular weight: 228.3 water solubility: 2500 mg/L K_{ow} = 63.1 pka = none (non-ionizable)

Table 2. Properties of the soils used in the experiments.

Property	Aksarben	Valentine	Rosebud
Sand (%)	11	88	40
Silt (%)	58	6	50
Clay (%)	31	6	10
organic matter (%)	2.2	0.6	2.7
pH	6.3	5.6	7.6
CEC (cmol+/kg)	20.3	8	22.2
K (% base saturation)	3.2	5.3	6.9
Mg (% base sat.)	23.6	12.4	4.8
Ca (% base sat.)	62.9	58.1	88.3

Table 3. HPLC conditions for analysis of parent compound and nitrosated products.

compound	wavelength (nm)	flow rate (mL/min)	mobile phase (water:acetonitrile)	column	retention time (min)	limitation (ppm)
atrazine	235	1.0	50:50	25 cm × 4.6 mm Keystone Betasil NA	6.3	0.0.033
NNAT	246	1.0	50:50	25 cm × 4.6 mm Keystone Betasil NA	9.6	0.1
HA	235	1.0	50:50	15 cm × 4.6 mm Acentis C18	9.7	0.18
DIA	220	0.8	50:50	15 cm × 4.6 mm Acentis C18	2.8	0.18
DEA	220	0.8	50:50	15 cm × 4.6 mm Acentis C18	3.7	0.19
simazine	230	1.0	50:50	25 cm × 4.6 mm Keystone Betasil NA	5.3	0.11
nitrosamazine	230	1.0	50:50	25 cm × 4.6 mm Keystone Betasil NA	7.5	0.1
cyanazine	234	1.0	50:50	25 cm × 4.6 mm Keystone Betasil NA	5.2	0.11
ametryn	246	1.0	80:20	25 cm × 4.6 mm Keystone Betasil NA	4.2	0.156

diuron	211	1.0	50:50	25 cm × 4.6 mm Keystone Betasil NA	5.4	0.156
linuron	211	1.0	50:50	25 cm × 4.6 mm Keystone Betasil NA	10.2	0.165
tebuthiuron	253	1.0	50:50	25 cm × 4.6 mm Keystone Betasil NA	4.6	0.195

RESULTS AND DISCUSSION

N-Nitrosoatrazine Formation

Formation in Solution. At pH 2 and 3, NNAT was found in solution within 10 h, but no NNAT was detected at higher pH during a 48-h experiment. The atrazine concentration decreased in solution at pH 2 (Figure 3). Rapid production of NNAT in solution at low pH was likely due to conversion of NO_2^- to HNO_2 (pka = 2.8) and reaction of subsequent nitrosating species with unprotonated atrazine (pka = 1.68). The HNO_2 is in equilibrium with dinitrogen trioxide (N_2O_3), a nitrosating species (Eq 1, (Williams, 2004). Under highly acidic conditions HNO_2 is also converted to $H_2NO_2^+$ (pka = 1.7; Riordan et al., 2005), which forms the strongly nitrosating NO^+.

We also screened for NNAT using the Eisenbrand cleavage method under acidic conditions (Eisenbrand et al., 1975). In this procedure, the nitrosamines are denitrosated with 3% HBr and color reagent is added. The solution will turn purple when the reagent reacts with NO^+. Although HPLC analysis confirmed the presence of NNAT, we did not see any color changes in our tests. This suggests limitations of the Eisenbrand test.

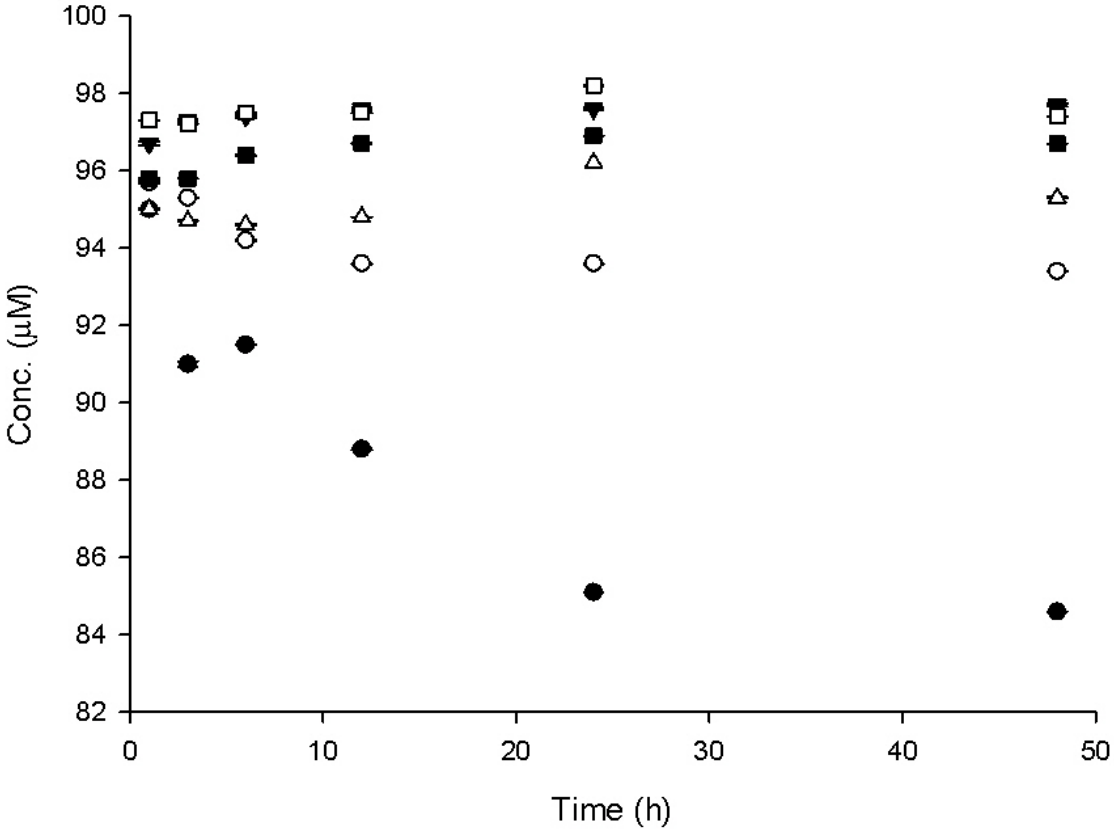


Figure 3. Loss of atrazine from reaction with nitrite in solution as affected by pH (● = pH 2, ○ = pH 3, ▼ = pH 4, Δ = pH 5, ■ = pH 6, □ = pH 7). Bars indicate standard deviations of the means; where absent bars fall within symbols.

Although NNAT was rapidly formed in solution at pH 2, it degraded to atrazine and hydroxyatrazine (HA) after 6 h (Figure 4). NNAT formed more slowly but was more stable at pH 3 than 2 (Figure 5). Atrazine initially decreased then increased as NNAT formed and was denitrosated in a 48-h experiment. This indicates that NNAT stability in solution decreases with increasing acidity.

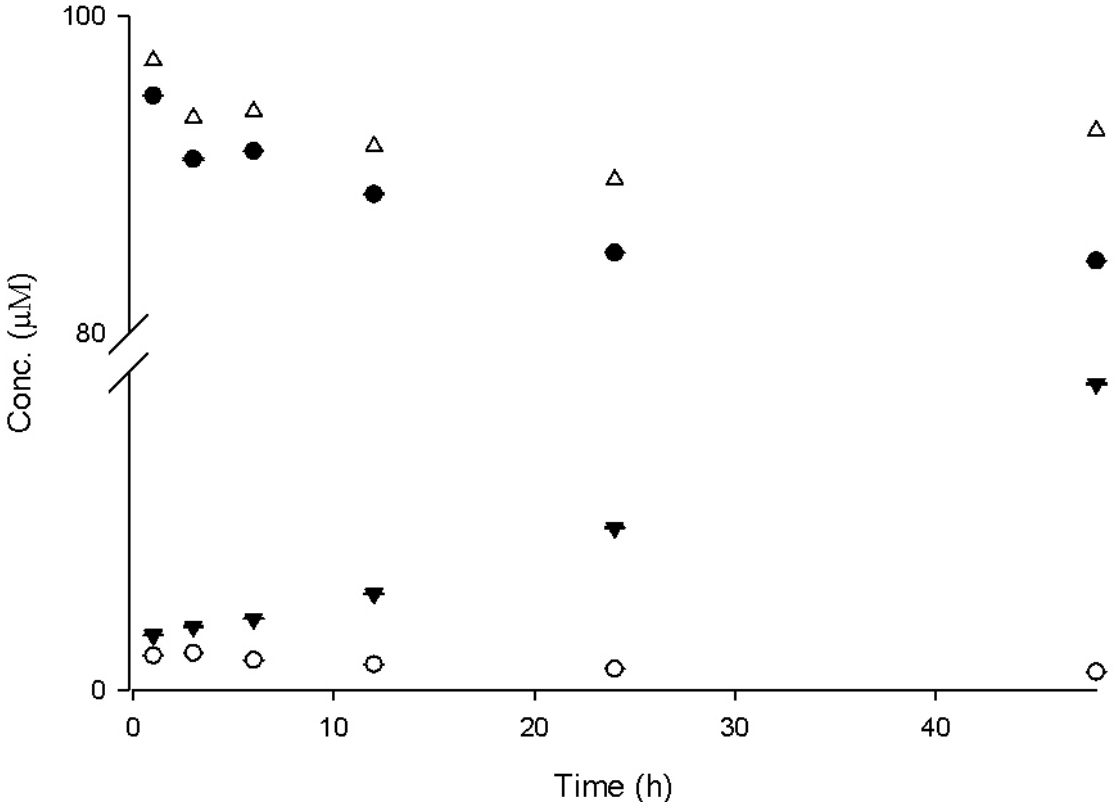


Figure 4. Formation of NNAT from reaction of atrazine with nitrite (1:4 molar ratio) in solution at pH 2 (● = atrazine at pH 2, ○ = NNAT at pH 2, ▼= hydroxyatrazine at pH 2, Δ = Total (atrazine + NNAT + HA)). Bars indicate standard deviations of the means; where absent bars fall within symbols.

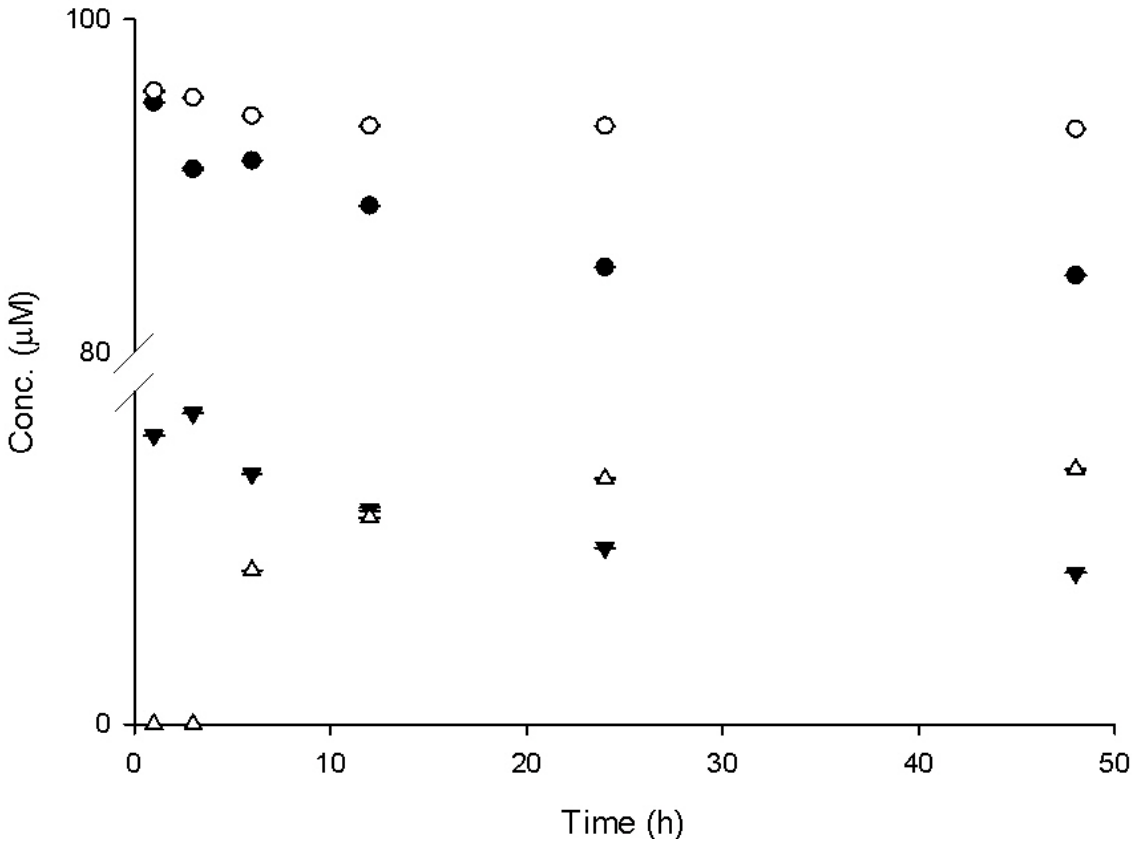


Figure 5. Formation of NNAT from reaction of atrazine with nitrite (1:4 molar ratio) in solution at pH 2 and 3 (● = atrazine at pH 2, ○ = atrazine at pH 3, ▼ = NNAT at pH 2, Δ = NNAT at pH 3). Bars indicate standard deviations of the means; where absent bars fall within symbols.

In a 14-d solution experiment (Figure 6), NNAT was formed only at pH 2 to 4. However, the addition of acetate resulted in NNAT formation at pH 5 to 7 and also appeared to increase NNAT stability (Figure 7). Cova (1986) similarly reported formation of *N*-nitrosocurzate in solution containing 350 mM acetate at pH higher than without acetate. Mirvish (1973) observed that thiocyanate (NCS^-) increased the nitrosation reaction ten-fold at pH 2.5. The acetate anion may increase the productivity of the reaction in a manner similar to NCS^-, which involves formation of

nitrosyl thiocyanate (ON·NCS) and subsequent reaction with the secondary amine (Eq. 11 and 12) (Mirvish, 1975).

$$HNO_2 + H^+ + NCS^- \rightleftharpoons ON{\cdot}NCS + H_2O \quad (11)$$

$$ON{\cdot}NCS + R_2NH \rightarrow R_2N{\cdot}NO + H^+ + NCS^- \quad (12)$$

$$d\,[R_2N{\cdot}NO]/dt = k'_x\,[HNO_2]\,[H^+]\,[NCS^-]\,[R_2NH] \quad (13)$$

$$rate = k'_x\,[HNO_2]\,[NCS^-]\,[R_2NH] \quad (14)$$

A similar mechanism may responsible for catalysis by the acetate anion. Such carboxylate ions also have been shown to catalyze formation of N_2O_3 (Hughs et al., 1958; Turney and Wright, 1959) (Eq. 15 and 16):

$$H_2NO_2^+ + {}^-OCOCH_3 \rightleftharpoons NO{\bullet}OCOCH_3 + H_2O \quad (15)$$

$$NO{\bullet}OCOCH_3 + NO_2^- \rightarrow N_2O_3 + {}^-OCOCH_3 \quad (16)$$

Masui et al. (1974) observed an increase in the rate of dimethylamine nitrosation with increasing acetate concentration. Thus the rate of nitrosation in the presence of acetate may be as described by Masui et al. (1974) (Eq 17):

$$d[R_2N{\cdot}NO]/dt = (k_{3OAc}\,[NO_2^-]_0^2 + k_{3OAc}[NO_2^-]_0^2[AcO^-] +$$

$$k_2^0[NO_2^-]_0 + k_{2OAc}[NO_2^-]\,[AcO^-])[amine]_0 \quad (17)$$

Fulvic acid also promoted NNAT formation at pH 4 and 5 (Figures 6 and 7). Weerasooriya and Dissanayake (1989) showed the rate of dibutylamine (DBA) nitrosation doubled when fulvic acid was present and nitrosation occurred under less

acidic conditions. This may be primarily due to the presence of more strongly acidic polycarboxlate groups in fulvic acid (pka ≤ 3.0; Leenheer et al., 1995a, 1995b), which would be expected to have a catalytic effect similar to acetate. Weerasooriya and Dissanayake (1989) reported that the nitrosation reaction rate depended on fulvic acid concentration and suggested that the fulvic acid lowered the activation energy of the amine-nitrosamine ion complex. Keefer and Roller (1973) also showed that nitrosation was catalyzed by formaldehyde, suggesting that the importance of carbonyl groups in the mechanism of catalysis. In addition, fulvic acids absorb energy over a large range of the electromagnetic spectrum and are known to generate free radicals, which may have a further catalytic effect(s). In contrast to fulvic acid, humic acid did not catalyze NNAT formation. Humic acid contains the higher molecular weight organic fractions and will precipitate under acidic conditions. At pH 2, rate of formation of NNAT was very similar in all treatments.

The presence of dissolved organic matter or fulvic acid had minimal effect on NNAT production or stability at pH 2 (Figure 6). NNAT reached a maximum concentration after 3 d, after which the atrazine concentration increased as NNAT gradually decreased. No NNAT formed in anaerobic solution at pH 4 or higher, while NNAT production continued in the aerobic treatments. These observations indicate the importance of oxygen in the reaction (Williams, 2004).

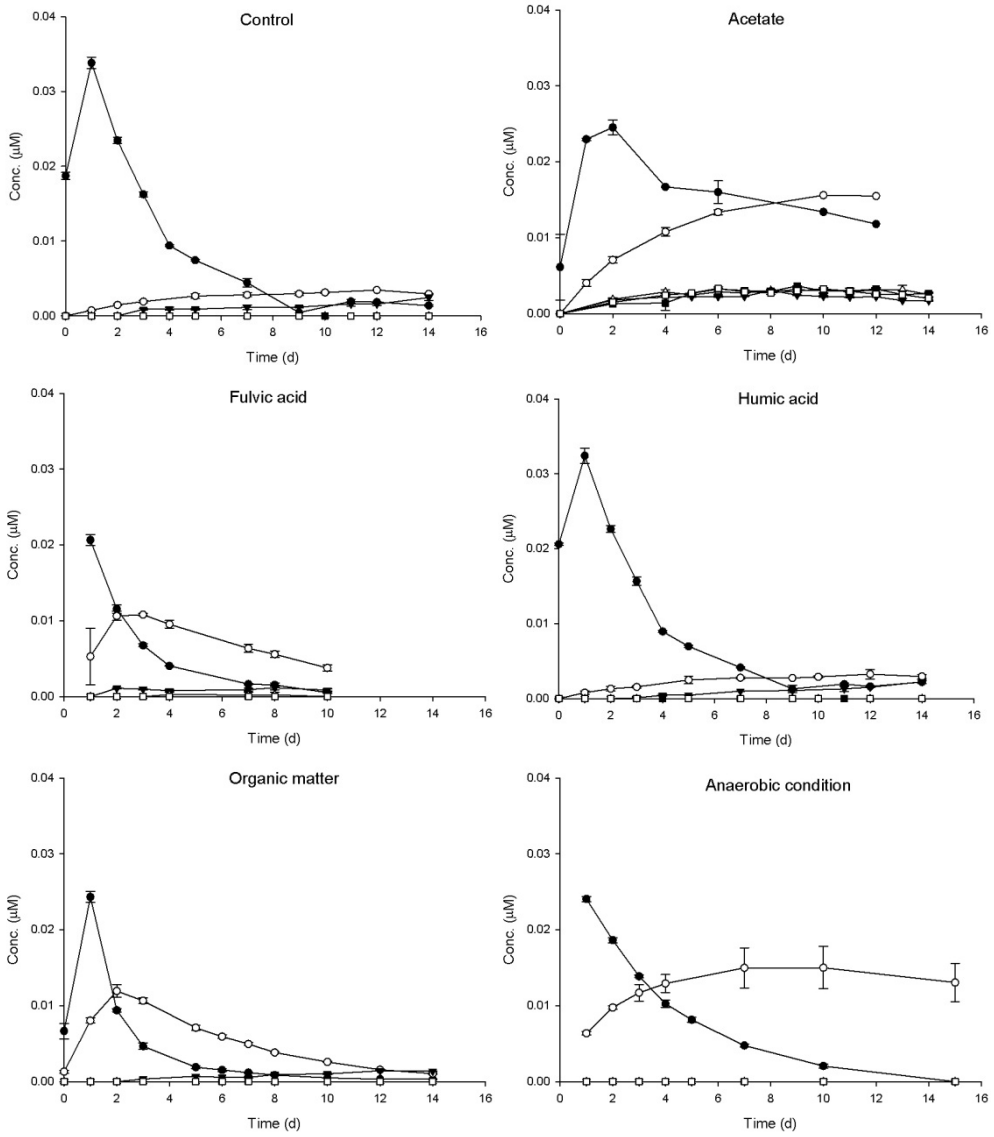


Figure 6. Formation of NNAT from reaction of atrazine and nitrite (1:4 molar ratio) with different solutes (100 mg/L) in solution pH 2 to 7 (● = pH 2, ○ = pH 3, ▼ = pH 4, Δ = pH 5, ■ = pH 6, □ = pH 7). Bars indicate standard deviations of the means; where absent bars fall within symbols.

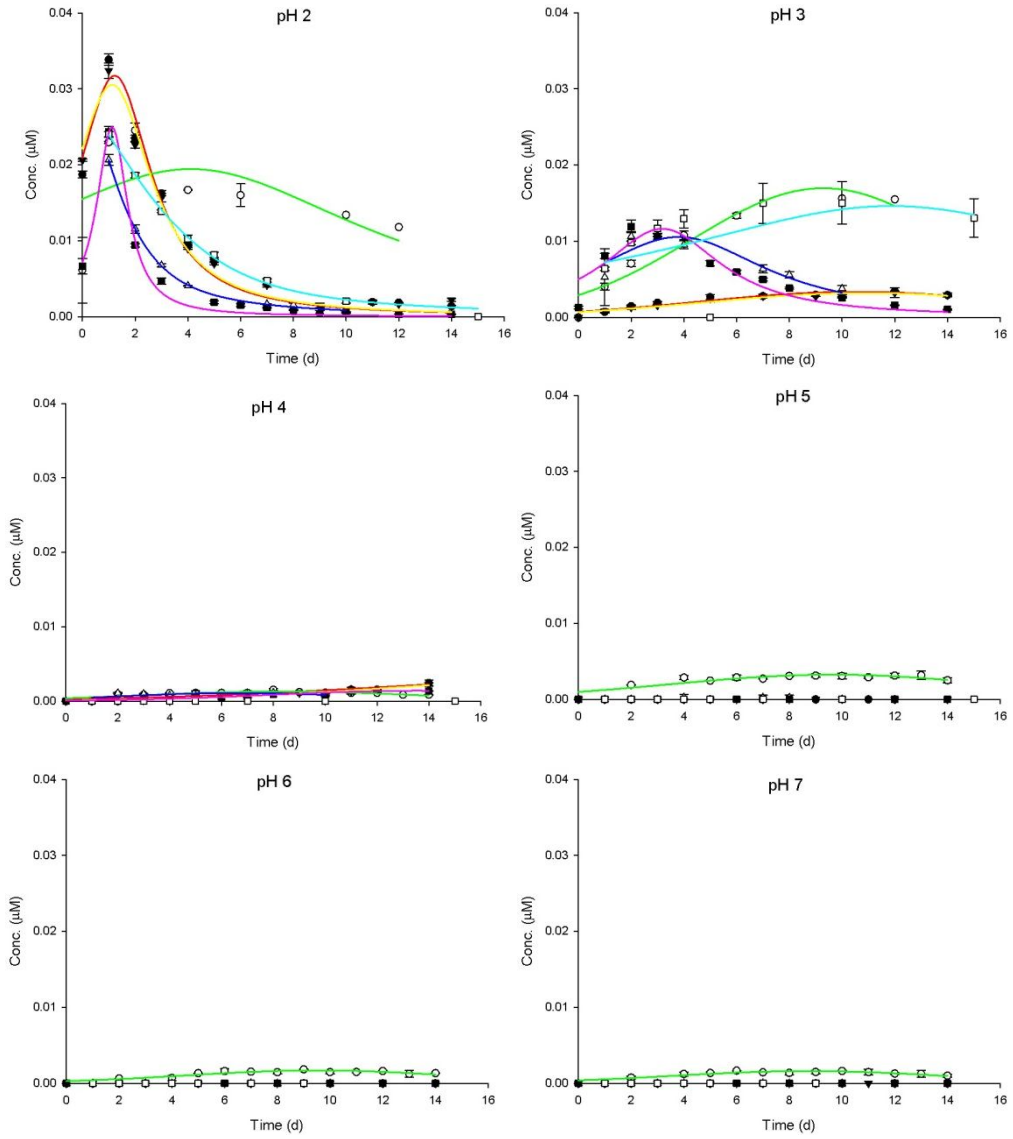


Figure 7. Formation of NNAT from reaction of atrazine and nitrite (1:4 molar ratio) in the presence of different (100 mg/L) solutes at pH 2 to 7 (● = no added solute, ○ = acetate added, ▼= humic acid added, Δ = fulvic acid added, ■ = organic matter extract added, □ = anaerobic conditions). Bars indicate standard deviations of the means; where absent bars fall within symbols.

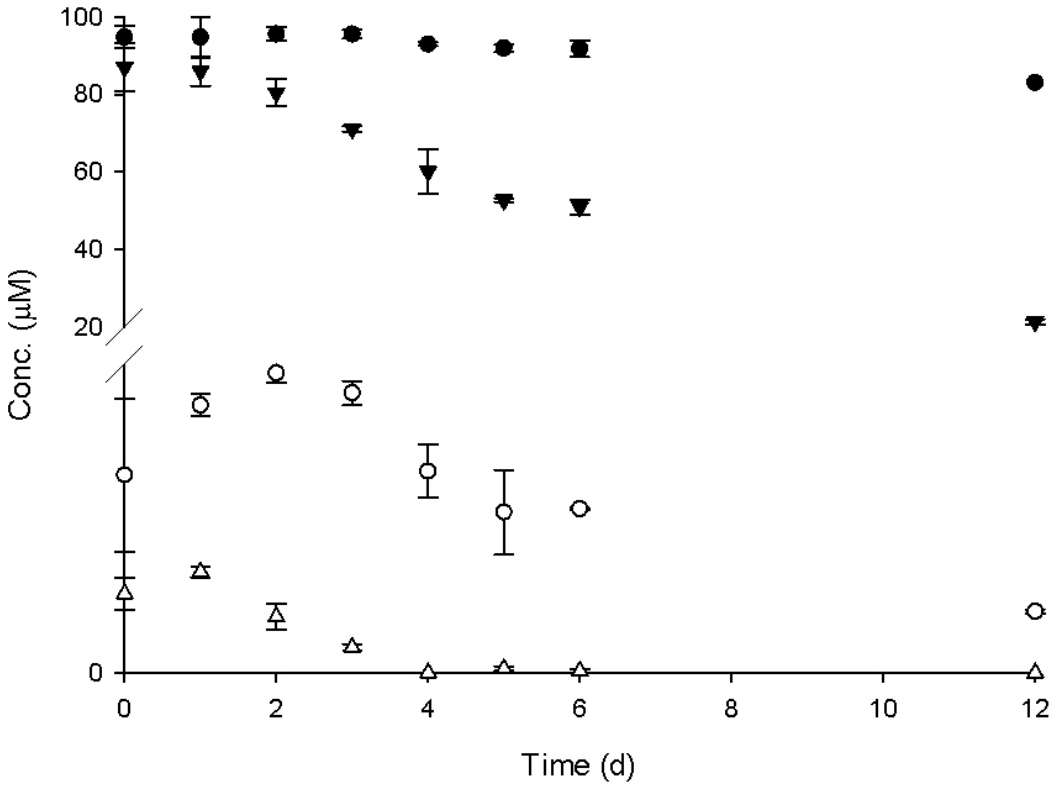


Figure 8. Formation of *N*-nitrososimazine from reaction of simazine with nitrite (1:4 molar ratio) in solution at pH 1 and 2 (● = simazine at pH 2, ○ = nitrososimazine at pH 2, ▼= simazine at pH 1, Δ = nitrososimazine at pH 1). Bars indicate standard deviations of the means; where absent bars fall within symbols.

Simazine (pka = 1.62) also formed *N*-nitrososimazine in solution containing nitrite (molar ratio 1:4) (Figure 8). It is likely that cyanazine (pka = 1.6) similarly formed *N*-nitrosocyanazine, but the product peak was not readily resolved from the parent under the HPLC conditions used. Nitrosation of cyanazine , as well as terbuthylazine, terbutryn, and terbumeton (all s-trazines with secondary amine moieties), was demonstrated in previous research (Janzowski et al., 1980; Cova et al., 1993; Wickenpflug and Richter, 1994; Cova et al, 1996).

Formation in a Soil-Water Slurry. During a 7-d experiment, NNAT was detected in the slurry at pH 2 to 5, but was not observed at pH 6 (Figure 9). This experiment showed that NNAT formed at a less acidic pH in the soil-water slurry than in water alone. The soil or dissolved solutes appear to be promoting the reaction, as observed in our solution experiments. NNAT concentration was greatest at pH 3, likely because more denitrosation was occurring at pH 2.

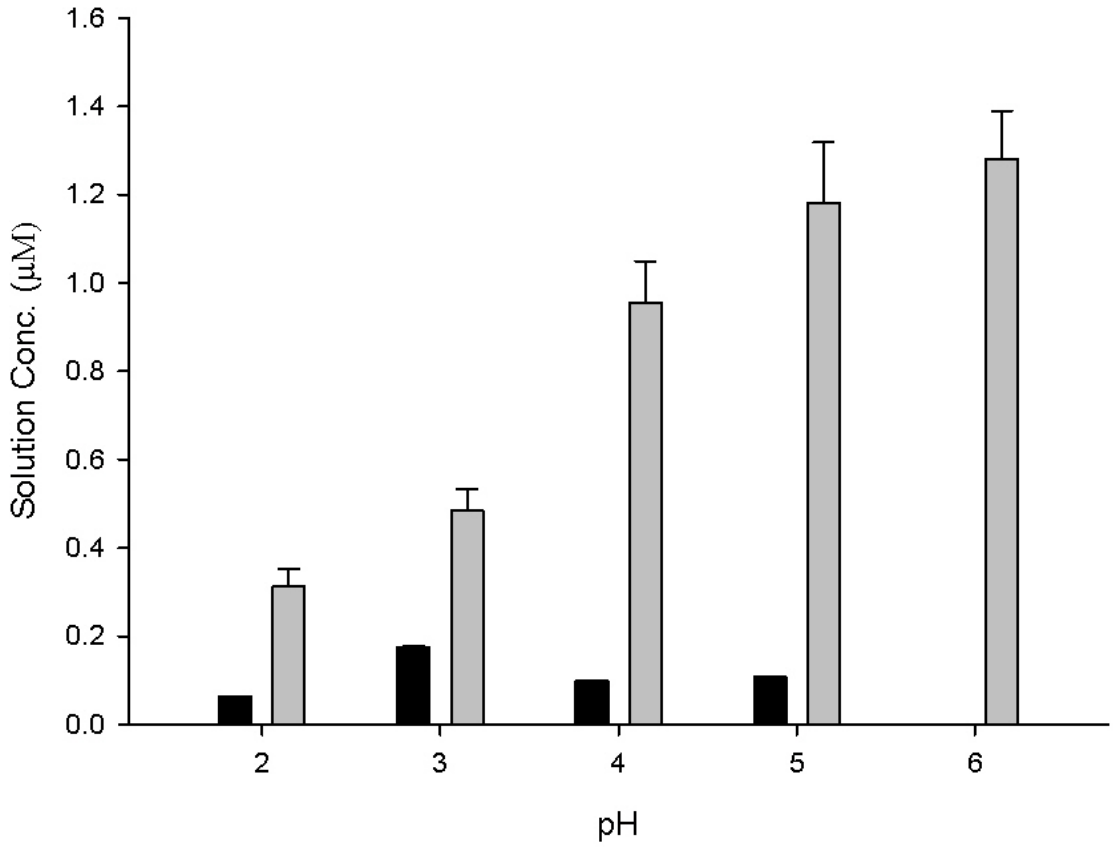


Figure 9. Formation of NNAT from reaction of atrazine with nitrite (1:156 molar ratio) in a soil-water slurry at pH 2 to 6 (■ = NNAT, ■ = atrazine). Bars indicate standard deviations of the means; where absent bars fall within symbols. Error bars indicate standard deviations of the means; where absent bars fall within solution concentration bars.

Formation in Soil. NNAT formed in soil after 7 d at pH 4 and after 14 d at pH 5, but no NNAT was found in soil at pH 6 and 7 (Figure 10). At pH 4, NNAT slowly degraded to atrazine, HA, and other compounds. NNAT formation in soil at pH 4 and 5 soil may be due to increased acidity at particulate surfaces and the presence of organic matter, which has been associated with nitrosamine formation (Mills and Alexander, 1976; Weerasooriya and Dissanyake, 1989). Padhye et al. (2010) showed that adsorption of DMA to activated carbon promoted its transformation to NDMA in the presence of oxygen, and similar promotion may be occurring at soil surfaces.

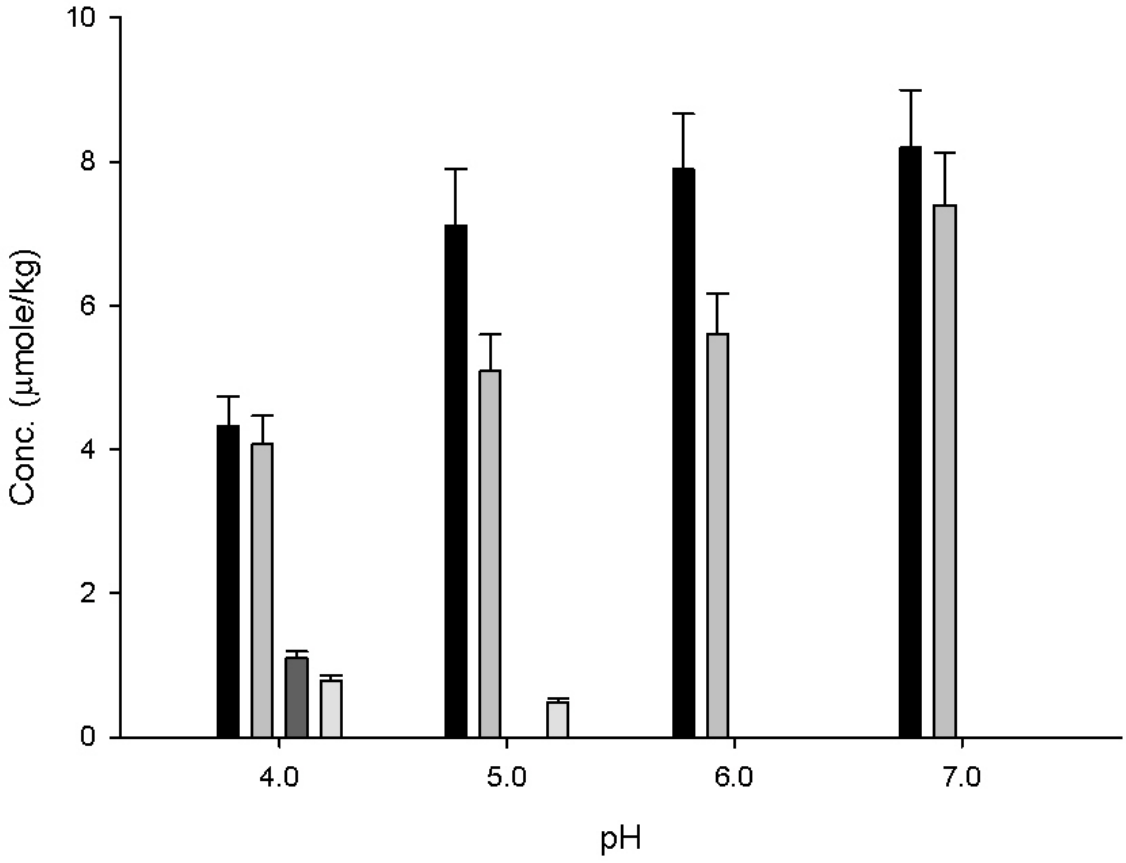


Figure 10. Formation of NNAT from atrazine and nitrite (1:156 molar ratio) in soil at pH 4 to 7 (■ = atrazine at 7 d, ■ = atrazine at 14 d, ■ = NNAT at 7 d, ■ = NNAT at 14 d). Error bars indicate standard deviations of the means.

To obtain more information about NNAT formation in soil at pH 4, the same experiment was repeated and concentrations monitored more frequently. NNAT reached a maximum at 7 d and the amount remained constant until 21 d (Figure 11). After 21 d the rate of denitrification may be exceeding nitrosation, resulting in loss of the nitrite and further slowing the reaction. Atrazine also may be degrading to other compounds. No NNAT was detected in pH 4 soil under oversaturated or anaerobic conditions (Figure 12). This indicates the importance of oxygen in the nitrosation reaction. Decomposition of nitrous acid in the absence of oxygen further slows nitrosation reactions (Williams, 2004).

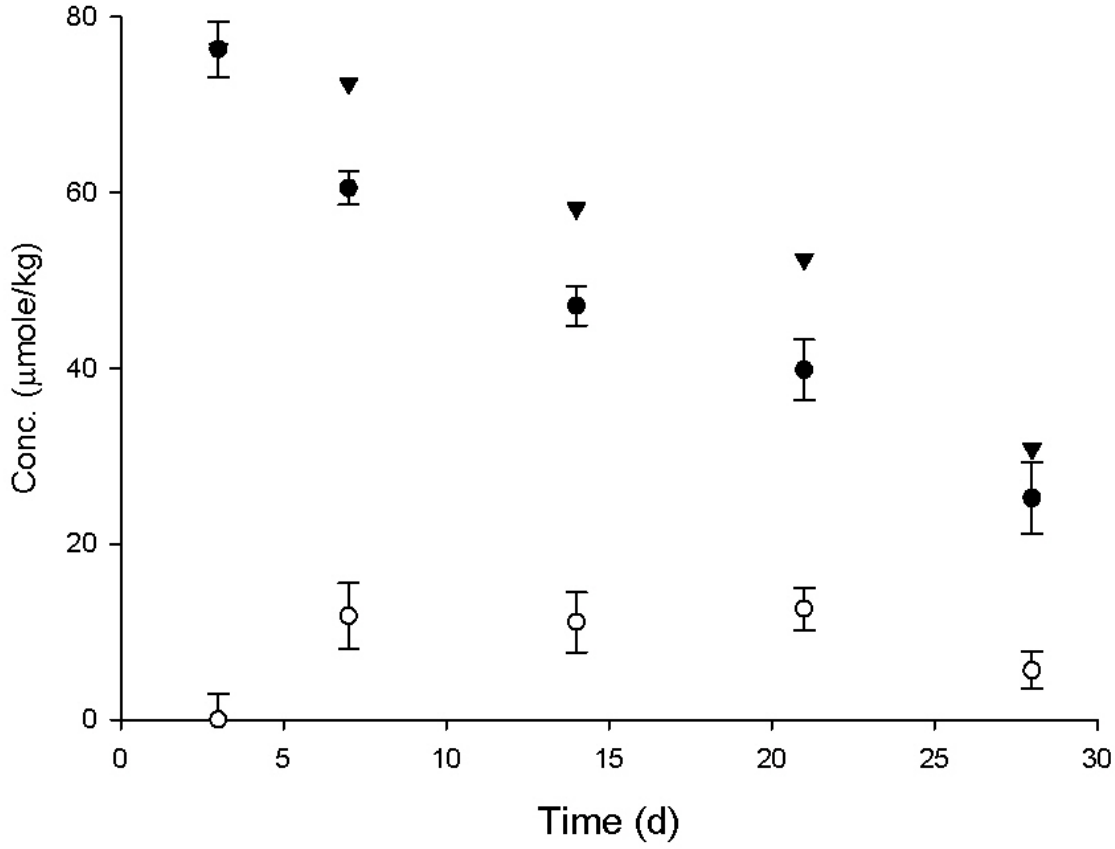


Figure 11. NNAT formation from reaction of atrazine with nitrite (1:156 molar ratio) in soil at pH 4 (● = atrazine, ○ = NNAT, ▼ = total (atrazine + NNAT)). Bars indicate standard deviations of the means; where absent bars fall within symbols.

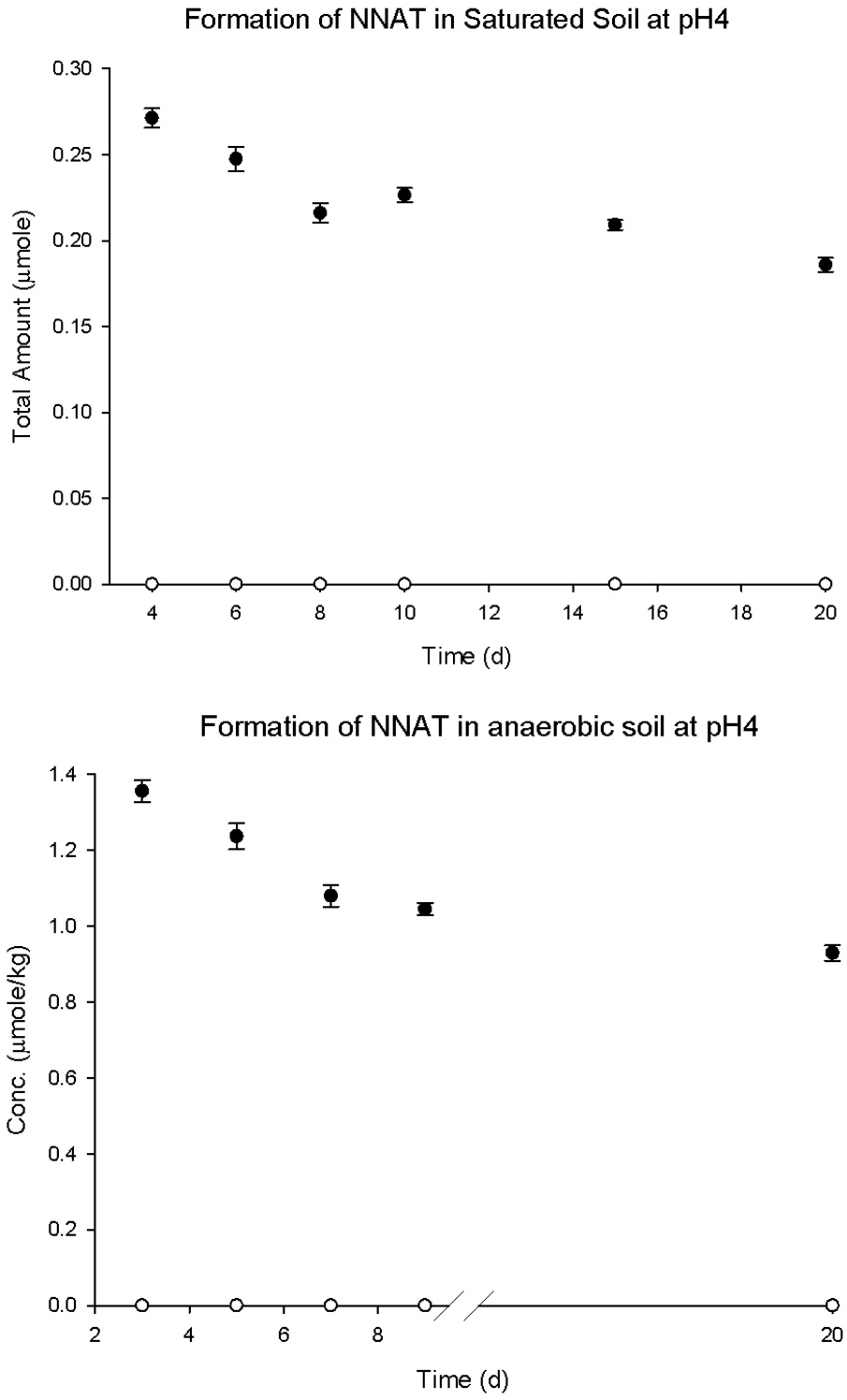


Figure 12. Formation of NNAT from atrazine and nitrite (1:156 molar ratio) under oversaturated and anaerobic conditions at pH 4 soil (● = atrazine, ○ = NNAT). Bars indicate standard deviations of the means; where absent bars fall within symbols.

No NNAT was found after incubating atrazine with nitrate for 1 month in soil at pH 2 to 4 and atrazine concentrations showed little change (Figure 13). Kearney et al. (1977) found decreases in atrazine concentrations under similar conditions at pH 2.5 (likely to HA), but they did not detect NNAT formation at pH 2.5 to 5.3. Although nitrate can be reduced to nitrite under reducing conditions, this was not likely under the aerobic conditions of the experiment. Denitrification only occurs in anaerobic environments where oxygen consumption exceeds the oxygen supply and where sufficient quantities of nitrate are present. Because the soil was incubated under aerobic conditions, production of nitrite from nitrate via denitrification would not occur.

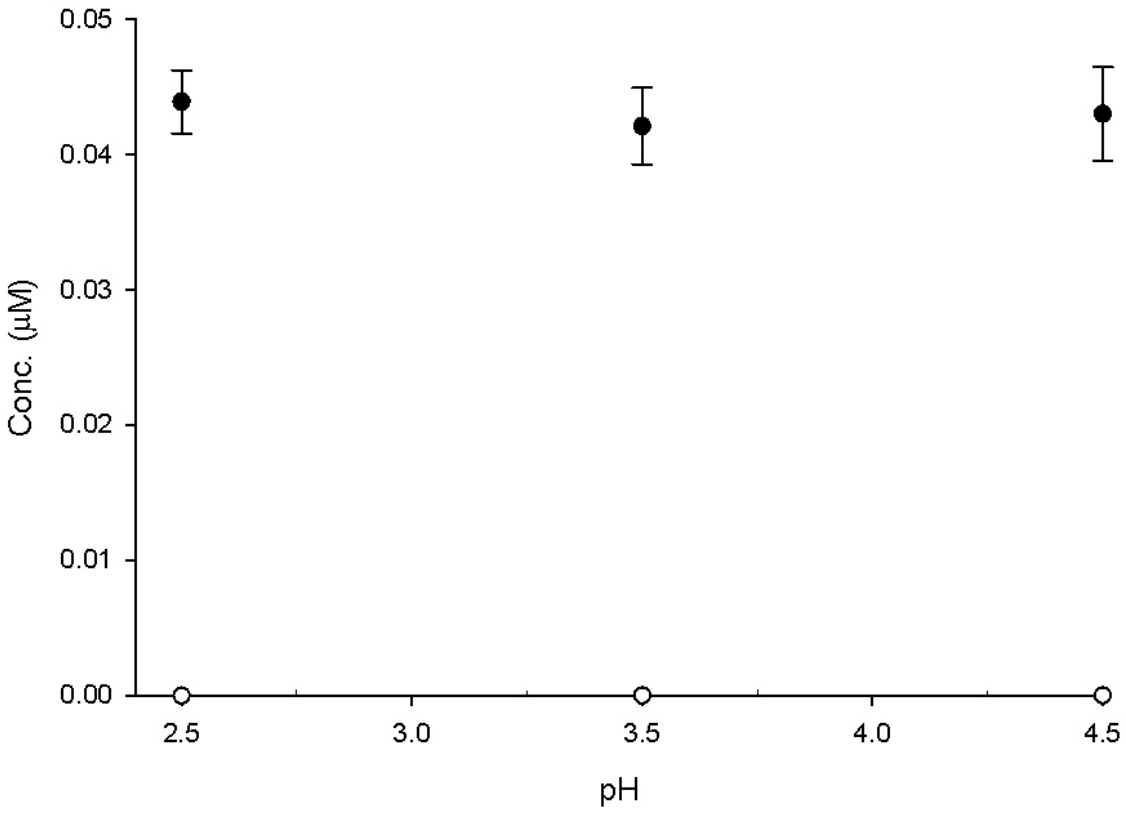


Figure 13. Formation of NNAT from reaction of atrazine with nitrate (1:156 molar ratio) in soil at pH 2 to 4 (● = atrazine, ○ = NNAT). Bars indicate standard deviations of the means; where absent bars fall within symbols.

Adsorption of *N*-Nitrosoatrazine in Soil

NNAT and atrazine adsorption and desorption were determined in Aksarben soil at different pH levels in a 2-d experiment (Figure 14). No effect of soil pH (ranging from 3 to 8) on NNAT and atrazine adsorption and desorption was observed. The K_{oc} values for atrazine and NNAT are given in Table 4. Adsorption K_d and K_{oc} values indicated that NNAT was more strongly adsorbed (average K_d = 5.93 and K_{oc} = 495) than atrazine (average K_d = 2.71 and K_{oc} = 123) average over all soil pH levels. Larger differences between adsorption and desorption K_d values indicated more hysteresis of NNAT than atrazine. These observations are consistent with the reported lower mobility of NNAT than atrazine in soil (Kearney et al., 1977). In contrast to NNAT, *N*-nitrosodimethylamine (NDMA) is reported to have low soil affinity and thus potentially higher mobility in soil (Gan et al., 2006).

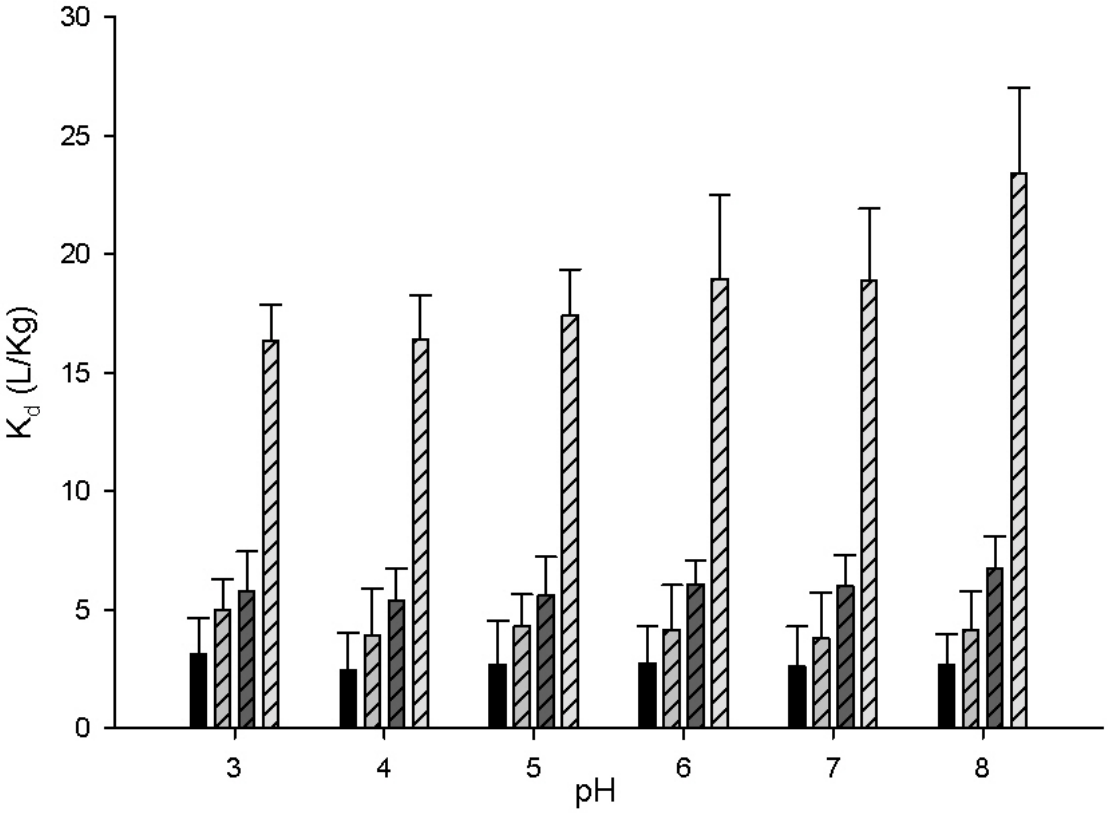


Figure 14. Atrazine and NNAT adsorption and desorption in soil at different pH levels (■ = atrazine adsorption, ■ = NNAT adsorption, ■ = atrazine desorption, ■ = NNAT desorption). Error bars indicate standard deviations of the means.

Table 4. Organic carbon partition coefficients (K_{oc}) for atrazine and *N*-nitrosoatrazine based on K_d measurements in a silt clay loam soil containing 2.2% organic matter.

pH	atrazine K_{oc} (±SD)	NNAT K_{oc} (±SD)
3	141 (±2)	481 (±2)
4	111 (±2)	448 (±1)
5	122 (±2)	466 (±2)
6	124 (±2)	508 (±1)
7	119 (±2)	503 (±1)
8	122 (±1)	561 (±1)
Average	123	495

NNAT and atrazine adsorption isotherms were obtained for Aksarben, Rosebud, and Valentine soils. Adsorption coefficients decreased in the order: NNAT in Aksarben > NNAT in Rosebud > atrazine in Aksarben > atrazine in Rosebud > NNAT in Valentine > atrazine in Valentine soil (Figure 15). NNAT showed greater adsorption than atrazine in the Aksaben silty clay loam and Rosebud silt loam soils. Adsorption of NNAT and atrazine was very low and similar in Valentine sandy soil. Soil texture affected NNAT and atrazine adsorption in soil. As expected, NNAT and atrazine adsorption was much less in the Valentine sand than in the Aksarben (silt clay loam) and Rosebud (silt loam) soils. We did not locate previous research on NNAT adsorption, but Mersie and Seybold (1996) reported adsorption coefficients decreasing in the order hydroxyatrazine > atrazine > desiopropylatrazine > desethylatrazine.

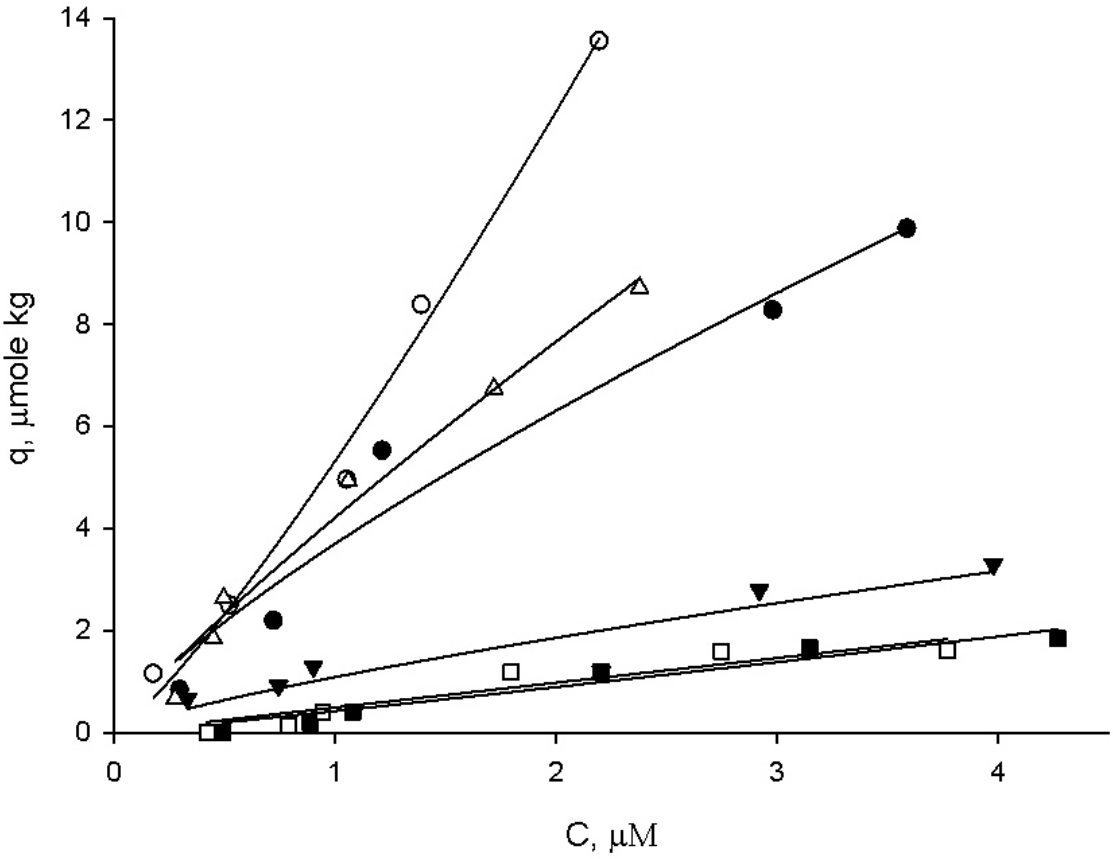


Figure 15. Adsorption Isotherms for NNAT and atrazine in three soils (● = atrazine on Aksarben, ○ = NNAT on Aksarben, ▼ = atrazine on Rosebud, Δ = NNAT on Rosebud, ■ = atrazine on Valentine , □ = NNAT on Valentine).

In bentonite clay, the adsorption coefficient for NNAT was larger than for atrazine (Figure 16). No NNAT or atrazine was detected in solution after equilibrating those compounds with bentonite clay treated with DDTMA or TAC, indicating that adsorption was further increased by coating the clay with organic material.

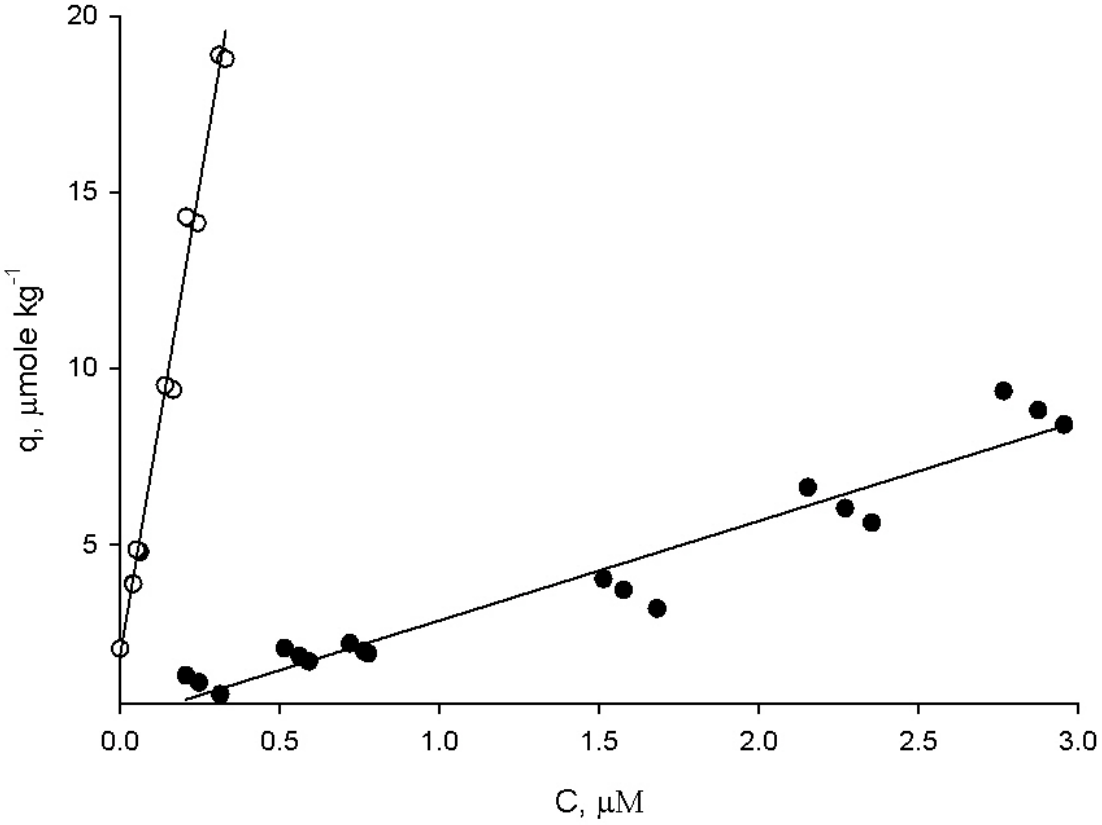


Figure 16. Adsorption isotherms for NNAT and atrazine on bentonite clay (● = atrazine, ○ = NNAT).

Degradation of *N*-Nitrosoatrazine in Solution and Soil

Degradation in Solution. In the absence of light, NNAT was relatively stable solution, with only small decreases in concentration in a 2-month experiment (Figure 17). The small increase in atrazine concentration during this period can be attributed to NNAT denitrosation.

Under light, NNAT rapidly degraded in solution (pH = 7) and atrazine concentration increased (Figure 18). Burns and Alliston (1971) also observed the photolytic decomposition of NDMA, nitrosodibutylamine (NDBA) and nitrosopiperidine (NNP) in solution at pH 4 and 9.2, and decomposition was faster at low pH. Lee et al. (2005a, 2005b) found that exposing aqueous solutions of NDMA to UV light resulted in production of dimethylamine (DMA), methylamine, nitrite, and nitrate. DMA production was favored at high NDMA concentrations and greatest at pH 4-5. Dissolved oxygen favored production of methylamine and nitrate, while *N*-methylformamide was produced under anoxic conditions. Interestingly, the production of NO_2^- (from denitrosation) further catalyzed NDMA degradation, which was attributed to attack of NO_2^- on the N-N bond in protonated NDMA to form DMA and N_2O_3 (Figure 19). Biggs and Williams (1975) had previously suggested that an *N*-protonated *N*-nitrosamine can be decomposed to the parent secondary amine by the attack of a nucleophile in an acidic aqueous solution, even in the absence of light.

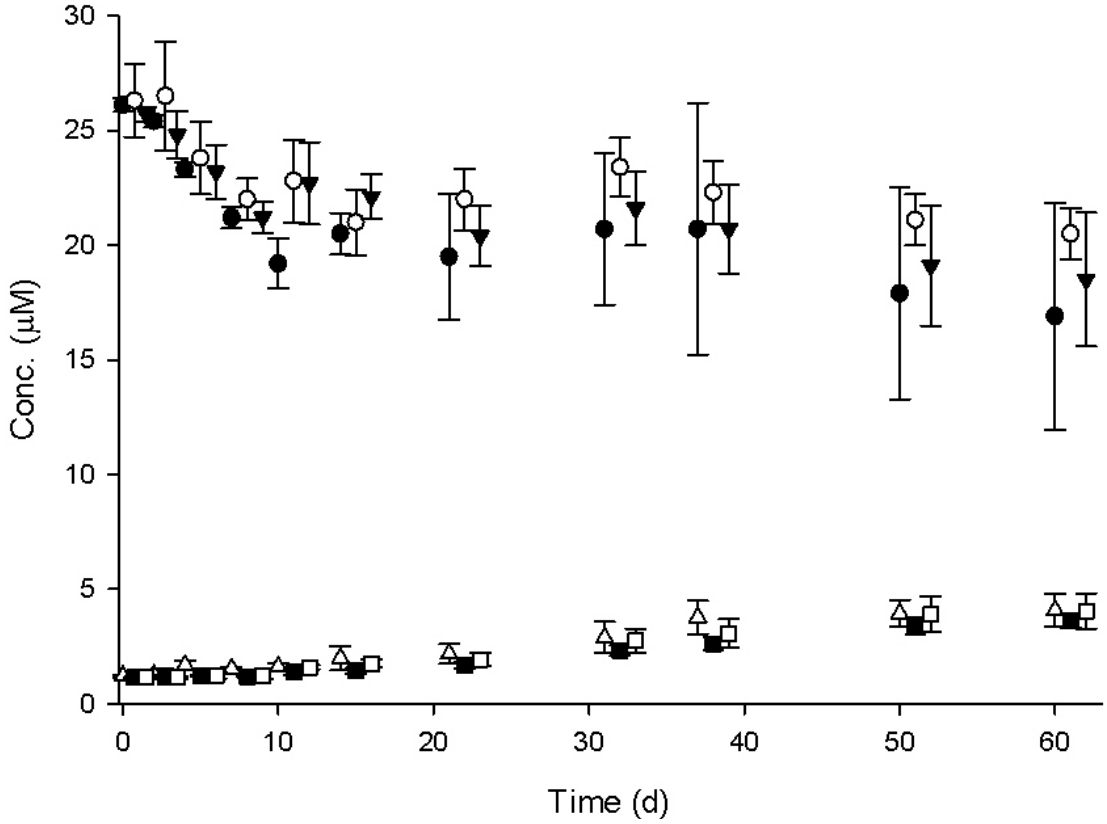


Figure 17. Stability of NNAT in solution at pH 4, 6, and 8 (● = NNAT at pH 4, ○ = NNAT at pH 6, ▼ = NNAT at pH 8, Δ = atrazine at pH 4, ■ = atrazine at pH 6, □ = atrazine at pH 8). Bars indicate standard deviations of the means; where absent bars fall within symbols.

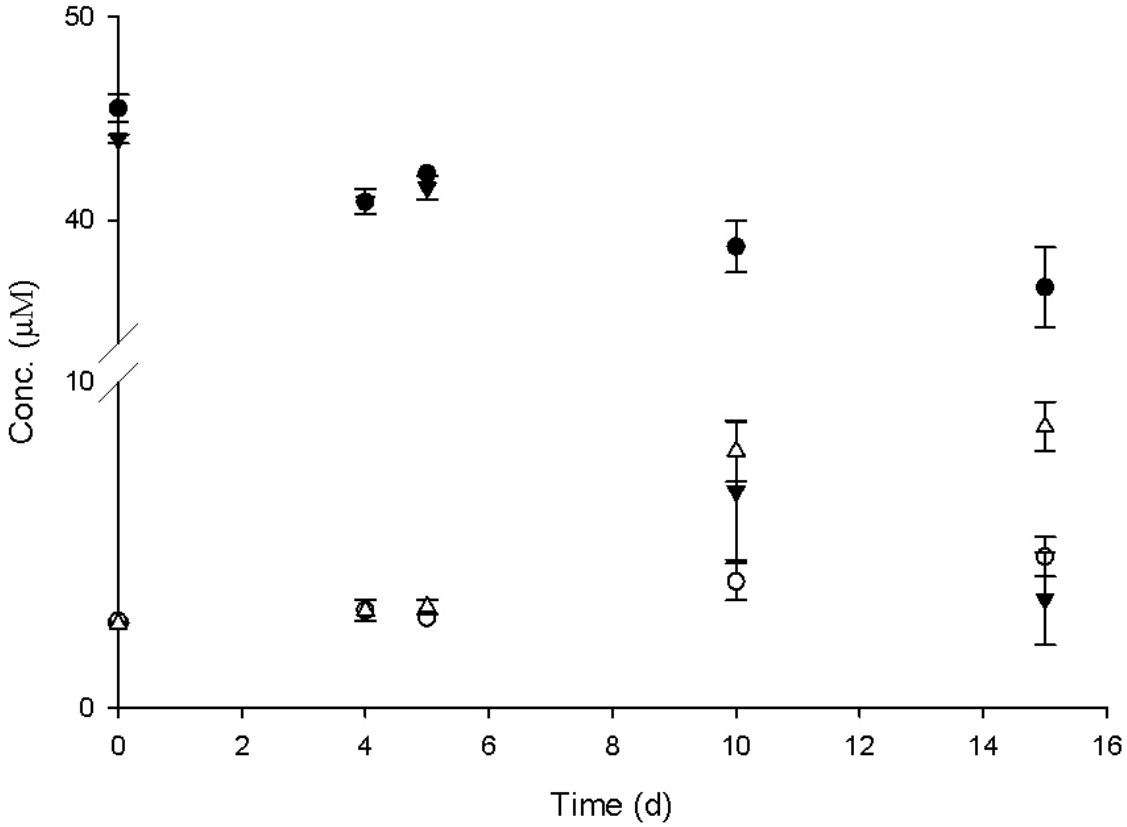


Figure 18. Stability of NNAT in solution (pH = 6.7) with and without light (● = NNAT without light, ○ = atrazine without light, ▼ = NNAT with light, Δ = atrazine with light). Bars indicate standard deviations of the means; where absent bars fall within symbols.

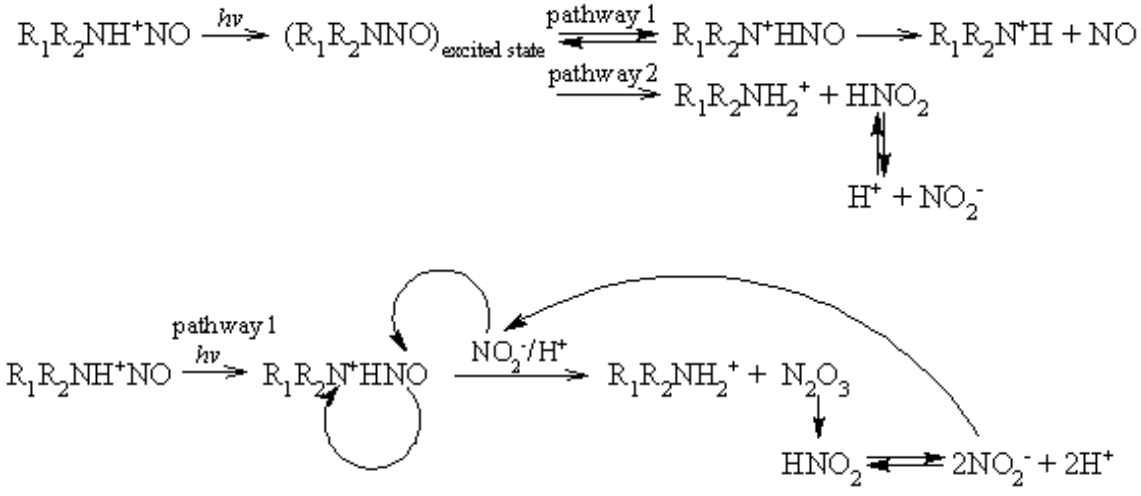


Figure 19. Two pathways of NDMA photolysis in solution (Lee et al. 2005a).

The rate of NNAT degradation was very similar in solution (netural pH) containing acetate, humic acid, fulvic acid, and dissolved organic matter, or under anaerobic conditions). Somewhat less atrazine was found in the absence of added solutes, which may be due to further degradation to hydroxyatrazine (Figure 20).

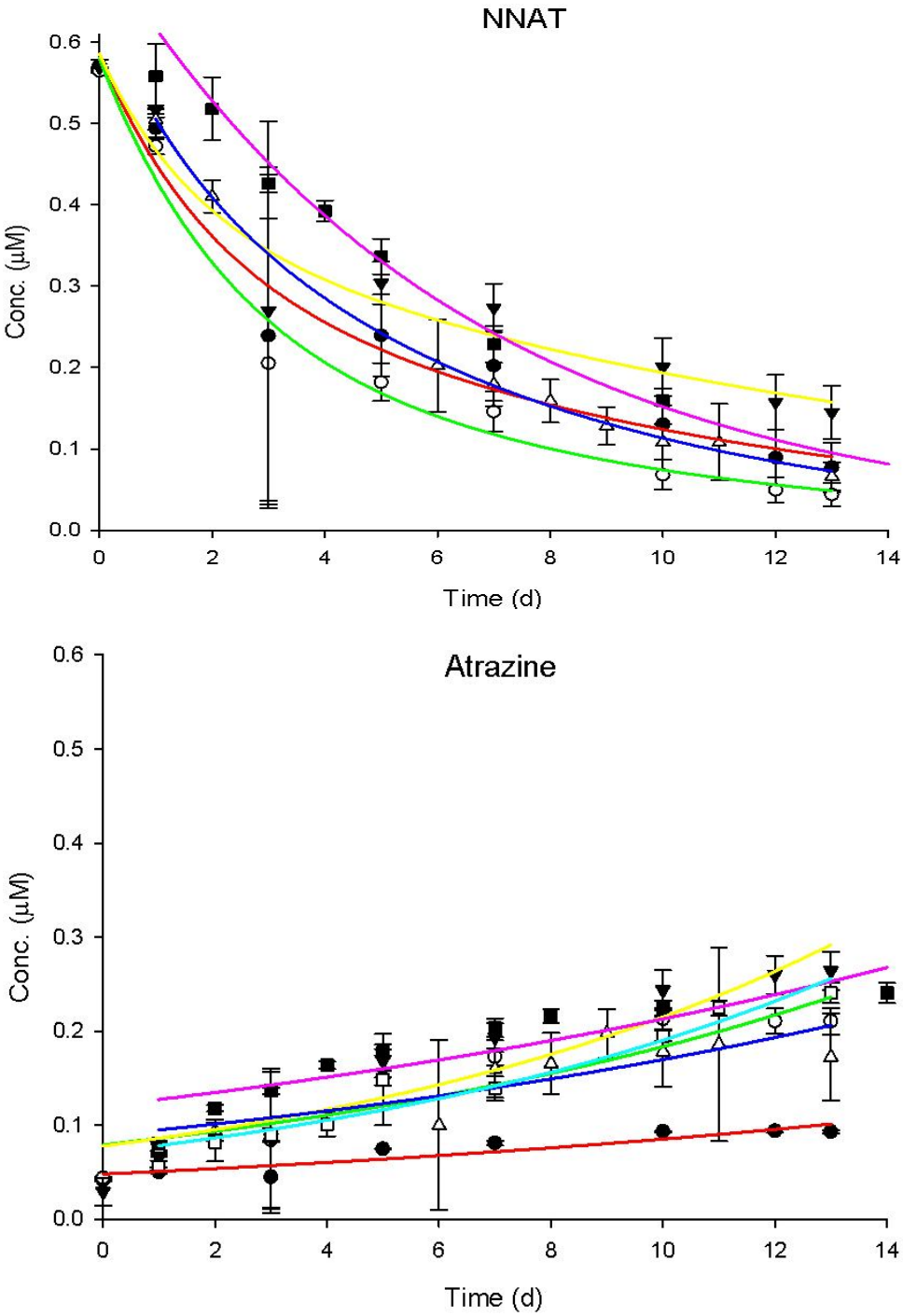


Figure 20 NNAT loss and atrazine detected during NNAT degradation in solution (● = no added solute, ○ = acetate added, ▼= humic acid added, Δ = dissolved organic matter added, ■ = fulvic acid added, □ = anaerobic condition). Bars indicate standard deviations of the means; where absent bars fall within symbols.

Degradation in Soil. In Aksarben soil (pH = 6.3), NNAT half -ife was about 9 d (Figure 21). NNAT denitrosated to atrazine and some hydroxyatrazine was detected with time. NNAT degradation in soil was similar at pH 6 and 7, with greater loss after 15 d at pH 4 (Figure 22). Some atrazine was detected as NNAT degraded, and the amount was somewhat greater at pH 4. Biodegradation is likely the major degradation mechanism at pH 6 and 7, and Yang et al. (2005) showed that soil organic matter was critical to the rate of NDMA degradation. Hydrolysis (denitrosation) may be contributing to the observed loss of NNAT at pH 4 and some of the resulting atrazine would be expected to form HA.

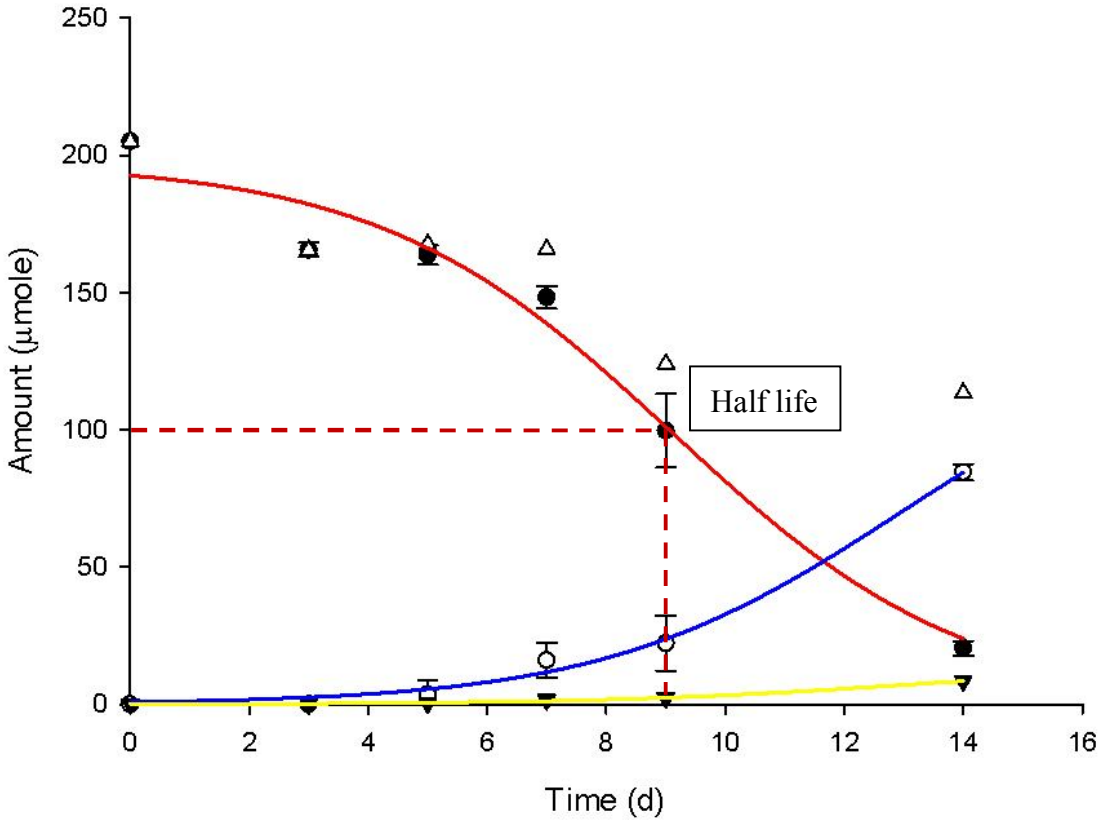


Figure 21. NNAT degradation in soil at pH 6.3 (● = NNAT, ○ = atrazine, ▼ = hydroxyatrazine, Δ = total amount). Bars indicate standard deviations of the means; where absent bars fall within symbols.

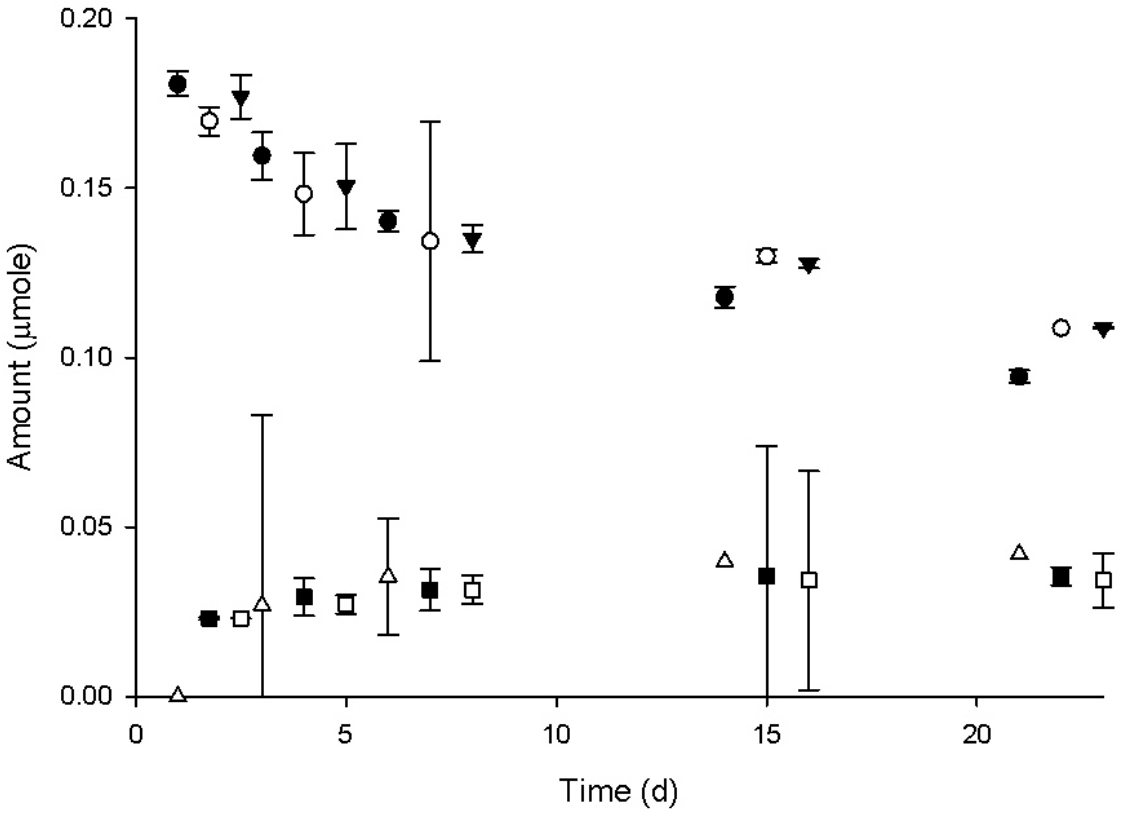


Figure 22. Degradation of NNAT in soil at pH 4, 6, and 7 (● = NNAT at pH 4, ○ = NNAT at pH 6, ▼= NNAT at pH 7, Δ = atrazine at pH 4, ■ = atrazine at pH 6, □ = atrazine at pH 7). Bars indicate standard deviations of the means; where absent bars fall within symbols.

Interestingly, NNAT more rapidly degraded in oversaturated soil than in moist soil (Figure 23). The large amount of water would be expected to facilitate NNAT desorption, increasing availability for degradation. The large amount of atrazine produced during NNAT degradation suggests that hydrolysis (denitrosation) may be a major mechanism. Denitrosation is promoted by removal of HNO_2 (Williams, 2004), which would result from conversion to NO_2 at the pH of the soil solution (6.3), and dilution due to the excess water present.

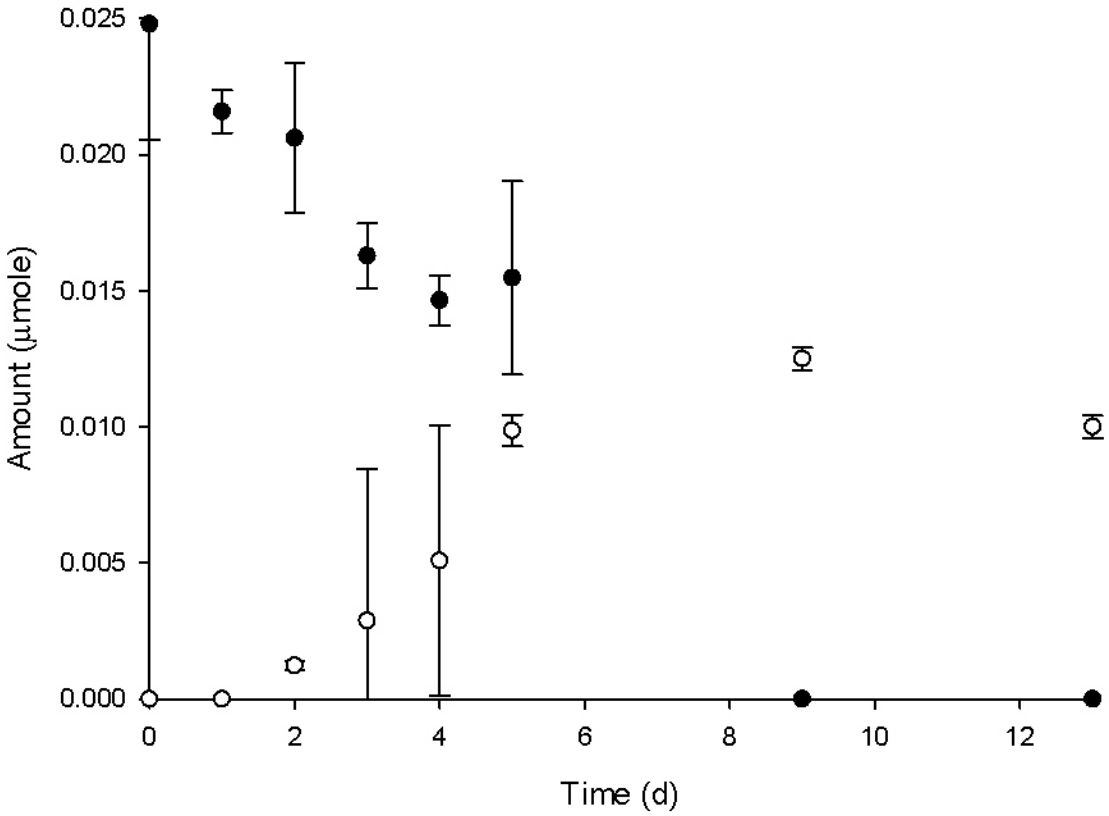


Figure 23. NNAT degradation in oversaturated soil (● = NNAT, ○ = atrazine). Bars indicate standard deviations of the means; where absent bars fall within symbols.

Nitrosation of Other Agrichemicals

Several other secondary amine and substituted urea pesticides (dethylatrazine, deisopropylatrazine, ametryn, diuron, linuron, and tebuthiuron) were evaluated for nitrosamine formation in solution containing $NaNO_2$ at pH 2 to 4, and in solution containing acetate, fulvic acid, humic acid, or dissolved organic matter. No nitrosamines were detected in any of the solutions using the Eisenbrand assay and HPLC analysis shaking for 7 d (Figure 24). These results show that not all pesticides with secondary amines will readily form nitrosamines. One of the possibilities is the higher pka (= 4.1) value of ametryn caused protonated ametryn is not a nuclephile compound to react with nitrosonium ion.

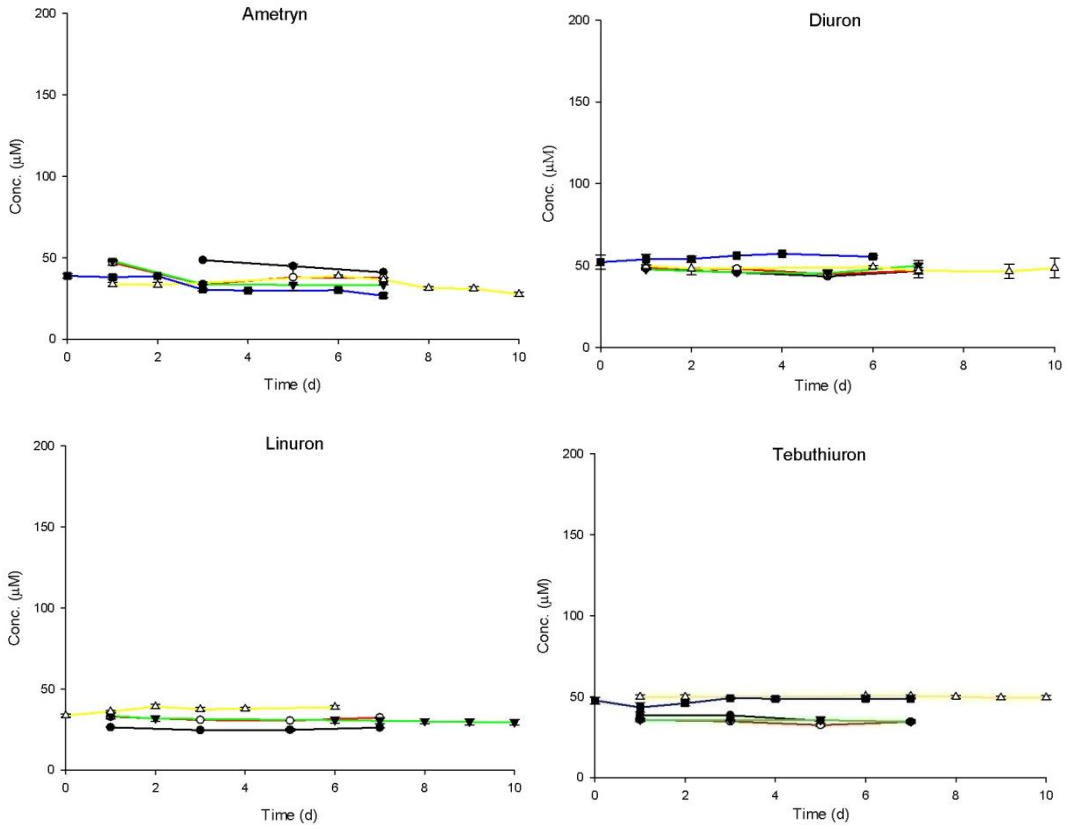


Figure 24. Results of nitrosation reactions with other secondary amine and substituted urea agrichemicals in solution (● = no dissolved solute added, ○ = acetate added, ▼ = humic acid added, Δ = dissolved organic matter added, ■ = fulvic acid added). Bars indicate standard deviations of the means; where absent bars fall within symbols.

CONCLUSIONS

Nitrosamines can form in agricultural soils treated with pesticides and veterinary pharmaceuticals containing secondary amine moieties and receiving heavy applications of nitrogen fertilizer. Atrazine is a widely used herbicide which can react with nitrite to form a potentially toxic nitrosamine product, *N*-nitrosoatrazine (NNAT).

Atrazine and nitrite formed NNAT in solution at pH 2 to 4, and in soil at pH 4 and 5. *N*-nitrososimazine also formed in acidic solution containing simazine and nitrite. NNAT formation rate and stability are affected by pH. The reaction is likely promoted at acidic pH because NO_2^- forms HNO_2, which becomes $H_2NO_2^+$, potentially producing the strongly nitrosating NO^+ species. However, in aqueous solution, HNO_2 is in equilibration with dinitrogen trioxide (N_2O_3), also a highly effective nitrosating agent. In addition, the presence of non-basic nucleophiles (X^-) can result in the formation of a third nitrosating species, XNO. The latter mechanism may explain catalysis of NNAT formation by acetate and fulvic acid in water at pH 5 to 7.

NNAT formation in soil at pH 4 and 5 may be due to increased acidity at particulate surfaces and promotion by organic matter. No NNAT was detected in pH 4

soil under oversaturated or anaerobic conditions, indicating the importance of oxygen in the nitrosation reaction. Decomposition of nitrous acid in the absence of oxygen further slows nitrosation reactions.

Adsorption K_d and K_{oc} values show greater adsorption of NNAT than atrazine at all agronomic soil pH levels. Soil texture affected NNAT and atrazine adsorption in soil. As expected, NNAT and atrazine adsorption was lower on the Valentine sand (which contains fewer adsorption sites) than on Aksarben silt clay loam and Rosebud silt loam soils. Larger desorption K_d values indicate greater hysteresis of NNAT than atrazine.

NNAT was relatively stable in solution, with only small decreases in concentration in a two-month experiment under dark conditions, but rapidly transformed to atrazine when exposed to light. In soil, NNAT degradation was similar at pH 6 and 7, with greater loss after 15 d at pH 4. Some atrazine was detected as NNAT degraded, and the amount was somewhat greater at pH 4. The half-life of NNAT in Aksarben silt clay loam was about 9 d, with degradation to atrazine and other compounds.

The information obtained from this research is important when evaluating atrazine fate and impacts in soil-water environment. NNAT may form when atrazine and nitrite are present in acidic waters and soils. The presence of dissolved organic

matter containing strongly acidic carboxylate groups (such as fulvic acid) can promote nitrosation reactions. Although oxygen is important for nitrosation, the presence of acetate, a fermentation product, will promote nitrosation under near-neutral conditions. Thus NNAT may be found in some locations where agrichemical runoff accumulates following application of atrazine and nitrogen fertilizers. NNAT may also be present in wetlands and riparian zones receiving agricultural runoff. The relatively high affinity for clay and organic matter would reduce NNAT availability for further movement, but it may persist in some ground waters. While exposure to light would promote NNAT degradation in surface water, sediment-adsorbed NNAT may be ingested by bottom feeders. The potential toxicity of NNAT warrants monitoring for the compound in environmental media where atrazine and nitrite are found.

LITERATURE CITED

Anastasio, C. and Chu, L. Photochemistry of nitrous acid (HONO) and nitrous acidium ion (H_2ONO^+) in aqueous solution and ice. *Environ. Sci. Technol.* **2009**, *43*, 1108-1114.

Arienzo, M.; Gan, J.; Ernst, F.; Qin, S.; Bondarenko, S.; Sedlak, D.L. Loss pathways of *N*-nitrosodimethylamine (NDMA) in turfgrass soils. *J. Environ. Chem.* **2006**, 35, 285-292.

Ayanaba, A.; Verstraete, W.; Alexander, M. Formation of dimethylnitrosamine, a carcinogen and mutagen, in soils treated with nitrogen compounds. *Soil Sci. Soc. Am. Proc.* **1973a**, *37*, 565-568.

Ayanaba, A.; Verstraete, W.; Alexander, M. Possible microbial contribution to nitrosamine formation in sewage and soil. *J. Nat. Cancer Inst.* **1973b**, *50*, 811-813.

Biggs, I. D. and Williams, D. L. H. Kinetics and mechanism of the Fischer-Hepp rearrangement and denitrosation. Part V. The mechanism of denitrosation. *J. Chem. Soc., Perkin 2.* **1975**, 107-111.

Black, C. A. Chemical fractionation of organic matter. *Method of Soil Analysis, Part 1-2.*; Amer. Soc. Agronomy, Inc.: Madison, Wisconsin, 1965; pp.1414-1417.

Burns, D. T. and Alliston, G.V. Some studies on the photolytic decomposition stage in the estimation of *N*-nitrosamines. *J. Fd Technol*. **1971**, *6*, 433-438.

Cova, D.; Arnoldi, A.; Griffini, A.; Rossini L. Formation in aqueous solution of *N*-nitroso curzate and the catalytic effect of some anions. *Environ. Contam. Toxicol*. **1986**, *37*, 672-676.

Dean-Raymond, D. and Alexander, M. Plant uptake and leaching of dimethylnitrosamine. *Nature*. **1977**, *262,* 394-396.

Eisenbrand, G..; Ungerer, O.; Preussmann, R. Formation of *N*-nitroso compounds from agricultural chemicals and nitrite. *IARC Scientific Publication* **1975**, *9*, 71-74.

Gan, J.; Bondarenko, S.; Ernst, F; Yang, W.; Ries, S.B.; Sedlak, D.L. Leaching of *N*-nitrosodimethylamine (NDMA) in turfgrass soils during wastewater irrigation. *J. Environ. Qual.* **2006**, *35*, 277-284.

Gatehouse, D.G. and Tweats, D.J. Mutagen formation after the addition of nitrite to normal human gastric juice. *Carcinogenesis*, **1982**, *3*(5), 597-598.

Gianessi, L. P. and Anderson, J. E. Pesticide Use in US Crop Production: 1995; Publisher: Washington, D.C.: National Center for Food and Agricultural Policy, 1995.

Gianessi, L. P.; Reigner, N. Pesticide Use in US Crop Production: 2002; Publisher: Washington, D.C.: National Center for Food and Agricultural Policy, 2006.

Hughs,E.D.; Ingold, C.K.; Ridd, J.H. Nitrosation, diazotisation, and deamination. Part 1. Principles, background, and method for the kinetic study of diazotization. *J. Phys. Chem.* **1958**, 58-65.

Janzowski, C.; R. Klein; Preussmann, R. Formation of *N*-nitroso compounds of the pesticides atrazine, simazine, and carbaryl with nitrogen oxides. *IARC Scientific Publication* **1980**, *31*, 329-339.

Kearney, P.C.; Oliver, J.E.; Helling, C.S.; Isensee, A.R.; Kontson, A. Distribution, movement, persistence, and metabolism of *N*-nitrosoatrazine in soils and a model aquatic ecosystem. *J. Agric. Food Chem.* **1977**, *25*, 1177–1181.

Keefer, L.K.; Roller, P.P. *N*-nitrosation by nitrite ion in neutral and basic medium. *Science*, 181, 1245-1247.

Khan, S.U. *N*-nitrosamine formation in soil from the herbicide glyphosate and its uptake by plants." *J. Am. Chem. Soc.* **1981**, *174*, 275-287.

Khan, S.U. and Young, J.C. *N*-nitrosamine formation in soil from the herbicide glyphosate. *J. Agric. Food Chem.* **1977**, *25*, 1430-1432.

Lai, C-H. and Chou, P-T. A theoretical study of thermodynamics and kinetics of nitrosamines: A potential NO carrier. *Theor. Chem. Account*. **2008**, *119*, 453-462.

Lee, C; Choi, W; Kim, Y.G.; Yoon, J. UV photolytic mechanism of *N*-nitrosodimethylamine in water: Dual pathways to methylamine versus dimethylamine. *Environ. Sci. Technol*. **2005a**, *39*, 2101-2106.

Lee, C; Choi, W; Kim, Y.G.; Yoon, J. UV photolytic mechanism of *N*-nitrosodimethylamine in water: Roles of dissolved oxygen and solution pH. *Environ. Sci. Technol*. **2005b**, *39*, 9702-9709.

Leenheer, J.A.; Weershaw, R.L.; Reddy, M. Strong-acid, carboxyl-group structure in fulvic acid from the Suwannee River, Georgia. 1. Minor structures. *Environ Sci. Technnol.* **1995a**, 29, 393-398.

Leenheer, J.A.; Weershaw, R.L.; Reddy, M. Strong-acid, carboxyl-group structure in fulvic acid from the Suwannee River, Georgia. 2. Major structures. *Environ Sci. Technnol.* **1995b**, 29, 399-405.

Masui, M.; Nakahara. H.; Ohmori, H.; Sayo H. Kinetic studies on the formation of dimethylnitrosamine. *Chem. Pharm. Bull.***1974**, *22*, 1846-1849.

Mallik, M.A.; Tesfai, K.; Pancholy, S.K. Formation of carcinogenic nitrosamines in soil treated with pesticides, and in sewage amended with nitrogen compounds. *Proc. Okla. Acad. Sci*. **1981**, *61*, 31-35.

Mersie, W. and Seybold, C. Adsorption and desorption of atrazine, deethylatrazine, deisopropylatrazine, and hydroxyatrazine on Levy wetland soil. *J. Agric. Food Chem*. **1996**, *44*, 1925-1929.

Mills, A. L. and Alexander, M. Factors affecting dimethylnitrosamine formation in samples of soil and water. *J. Environ. Qual*. **1976**, *5*, 437-440.

Mirvish, S.S. Formation of *N*-nitroso compounds: Chemistry, kinetics, and *in vivo* occurrence. *Toxicol. Appl. Pharmacol*. **1975**, *31*, 325-351.

Mirvish, S.S.; Gannett, P.; Babcook, D.M.; Williamson, D.; Chen, S.C.; Weisenburger, D.D. *N*-Nitrosoatrazine: Synthesis, kinetics of formation, and nuclear magnetic resonance spectra and other properties. *J. Agric. Food Chem.* **1991**, *39*, 1205-1210.

Oliver, J.E.; Kontson, A. Formation and persistence of *N*-nitrosobutralin in soil. *Bull. Environ. Contam. Toxicol.* **1978**, *20*, 170-173.

Oliver, J.E.; Kearney, P.C.; Kontson, A. Degradation of herbicide-related nitrosamines in aerobic soils. *J. Am. Chem. Soc.* **1979**, *27*, 887-891.

Padhye, L.; Wang, P.; Karanfil, T.; Huang, C-H. Unexpected role of activated carbon in promoting transformation of secondary amines to *N*-nitrosamines. *Environ. Sci. Technol.* **2010**, *44, 4161-4168*.

Pancholy, S.K. Formation of carcinogenic nitrosamines in soils. *Soil Biol. Biochem.* **1978**, *10*, 27-32.

Riordan , E.; Minogue, N.; Healy, D.; O'Driscoll, P.; Sodeau, J.R. Spectroscopic and optimization modeling study of nitrous acid in aqueous solution. *J. Phys. Chem.* **2005**, *109*, 779-786.

Rostkowska, K.; Zwierz, K.; Rozanski, A.; Moniuszko-Jakoniuk, J.; Roszczenko, A. Formation and metabolism of *N*-nitrosamines. *Polish J. Environ. Studies*, **1998**, *7*(6) 321-325.

Sithole, B.B.; Guy, R.D. Interactions of secondary amines with bentonite clay and humic materials in dilute aqueous systems. *Environ. Int.* **1985**, *11*, 499-504

Tate, R.L.; Alexander, M. Formation of dimethylamine and diethylamine in soil treated with pesticides. *Soil Sci.* **1974**, *118,* 317-321.

Tate, R.L.; Alexander, M. Stability of nitrosamines in samples of lake water, soil, and sewage. *J. Natl. Cancer Inst.* **1975**, *54*, 327-330.

Trevisan, M.; Graviani, E.; Del Re, A.M.; Arnoldi, A.; Bassoli, A.; Cova, D.; Rossignoli, A. Formation of *N*-nitrosoterbuthylazine and *N*-nitrosoterbutryn in a model system of soil water. *J. Agric. Food Chem.* **1998**, *46*, 314–317.

Turjanski, A.G.; Leonik, F.; Estrin, D.A.; Rosenstein, R.E.; Doctorovish, F. Scavenging of NO by melatonin. *J. Am. Chem. Soc*. **2000**, *122*, 10468-10469.

Turney, T.A. and Wright, G.A. Nitrous acid and nitrosation. *Chem. Rev*. **1959**, *59*, 497-513

USEPA. Atrazine, Simazine and Cyanazine; Notice of Initiation of Special Review. **1994**, Fed. Reg. *59*, 60412-60443.

Weed Science Society of America. *Herbicide handbook*, Ninth ed., S.A. Senseman, ed. Weed Science Society of America: Lawrence, KS, **2007**.

Weerasooriya, S.V.R.; Dissanayake, C.B. The enhanced formation of *N*-nitrosamines in fulvic acid mediated environment. *Toxicol. Environ. Chem*. **1989**, *25,* 57–62.

Williams, D.L.H. *Nitrosation reactions and the chemistry of nitric oxide,* First ed., Elsevier: London, **2004**.

Wolfe, N.L.; Zepp, R.G.; Gordon, J.A.; Fincher, R.C. *N*-nitrosamine formation from atrazine. *Bull. Environ. Contam. Toxicol.* **1976**, *15*, 342-347.

Yang, W.C.; Gan, J.; Liu, W.P.; Green, R. Degradation of *N*-nitrosodimethylamine (NDMA) in landscape soils. *J. Environ. Qual.* **2005**, *34*, 336-341.

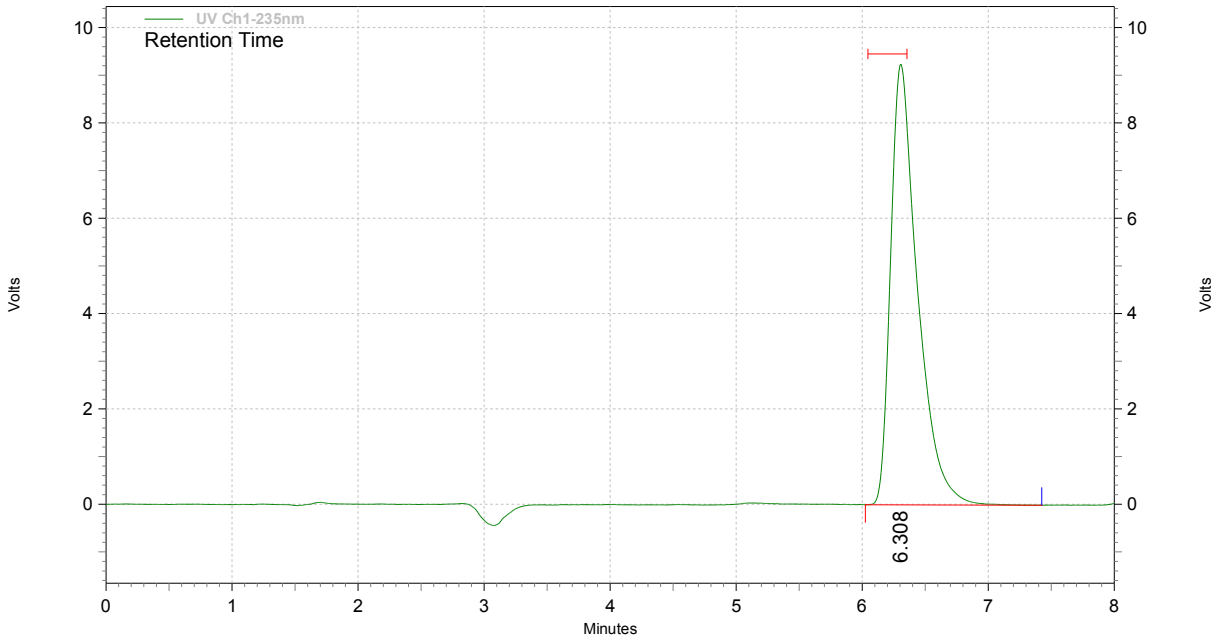
Young, J.C.; Khan, S.U.; Marriage, P.B. Fluorescence detection and determination of glyphosate via its *N*-nitroso derivative by thin-layer chromatography *J. Agric. Food Chem.* **1977**, *25*, 918–922.

Zwickenpflug, W.; Richter, E. Synthesis and occurrence of nitrosated cyanazine in soil. *J. Agric. Food Chem.* **1994**, *42*, 2333-2337.

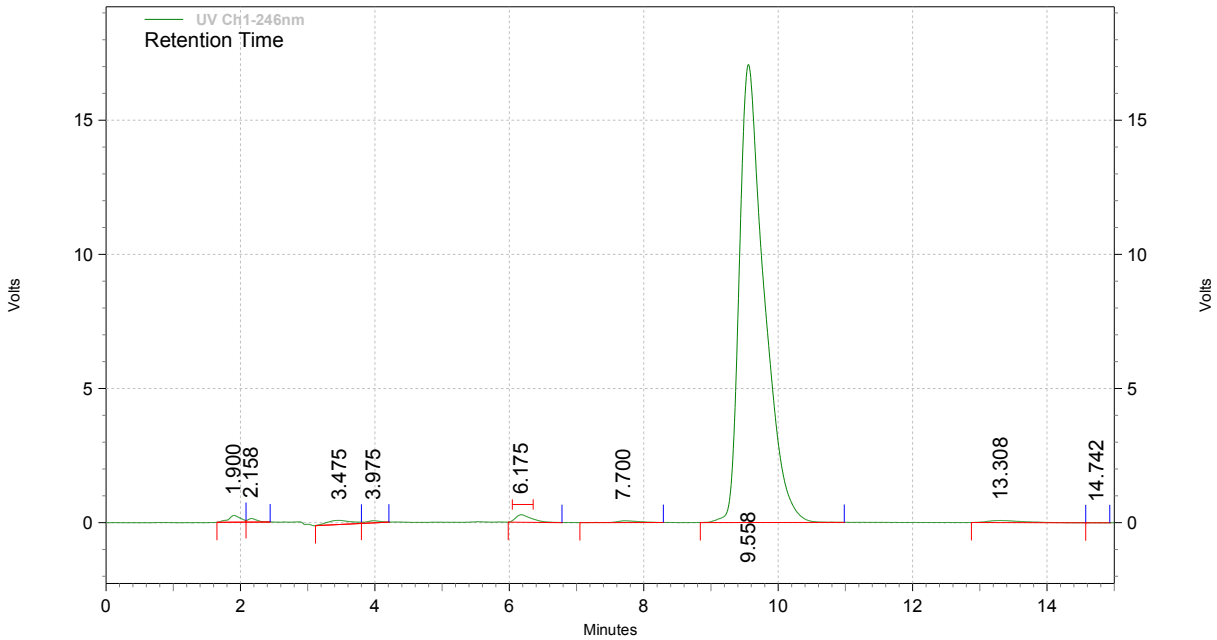
APPENDIX

Sample HPLC Chromatograms of Analyte Standards

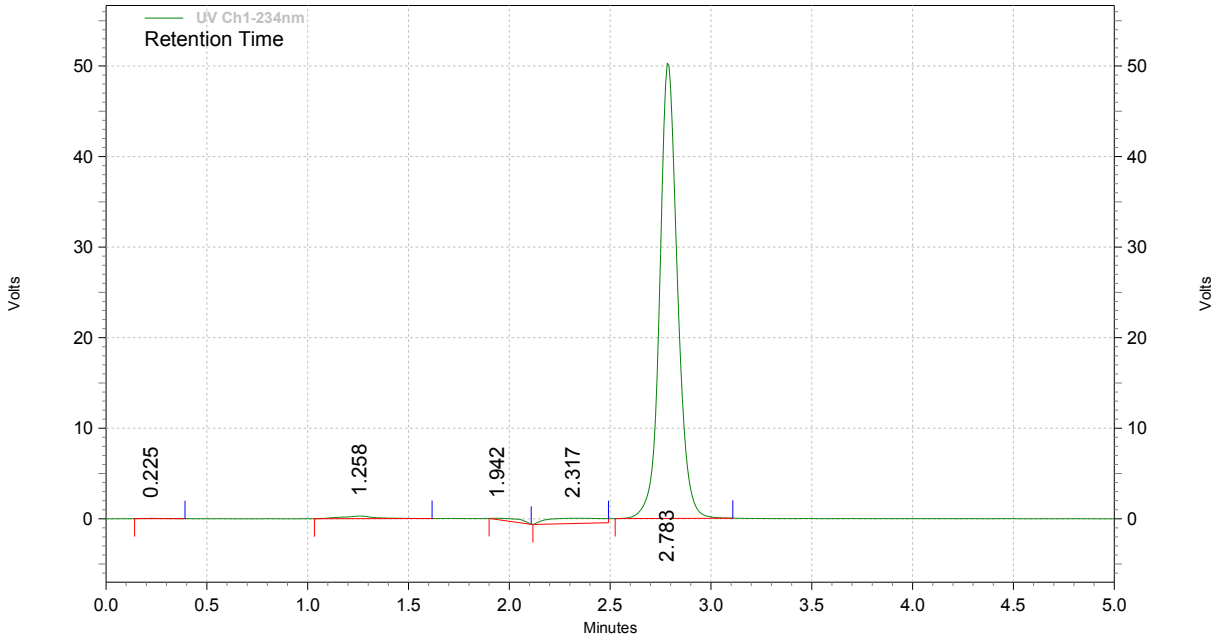
Atrazine 3.3 mg/L



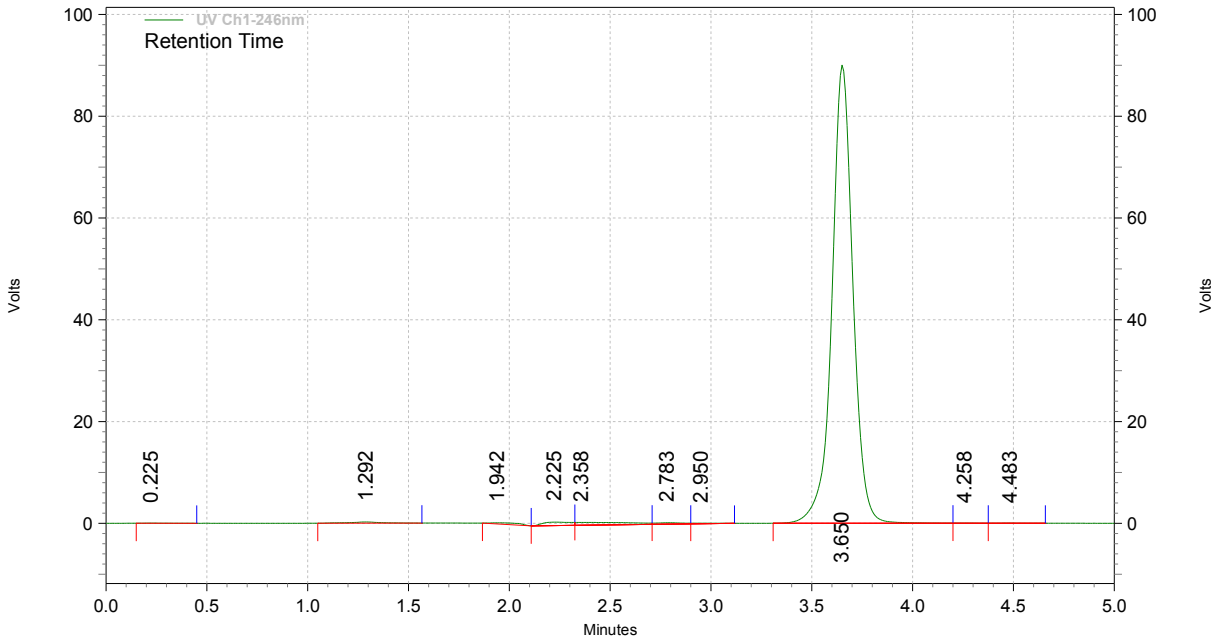
NNAT 5 mg/L



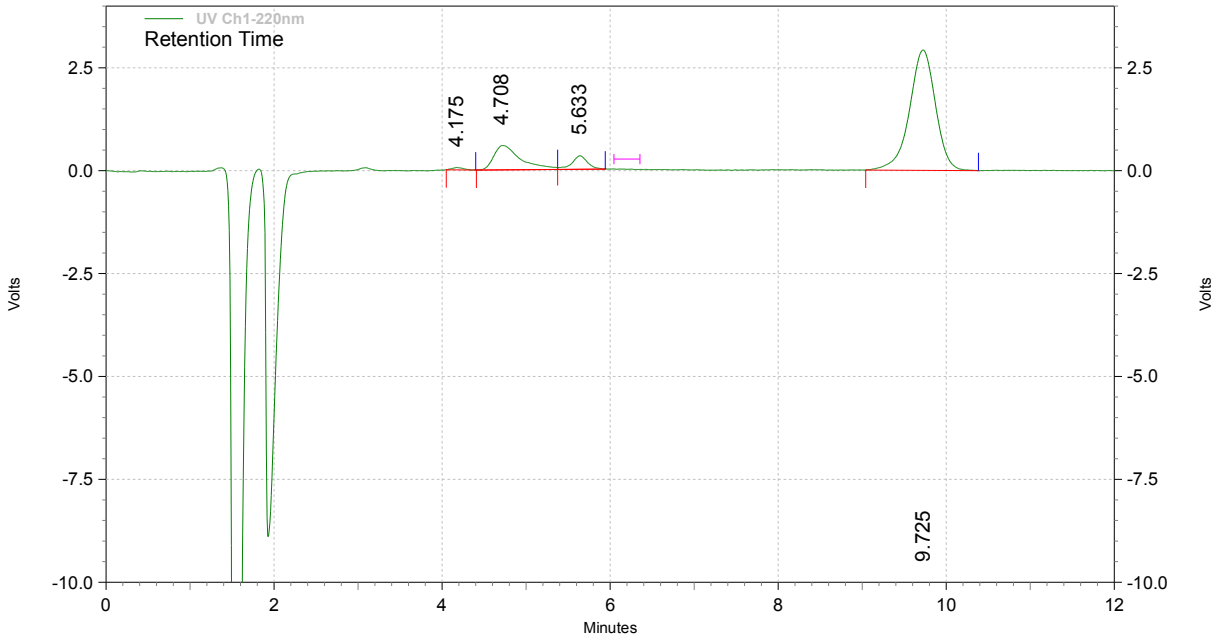
DIA 7 mg/L



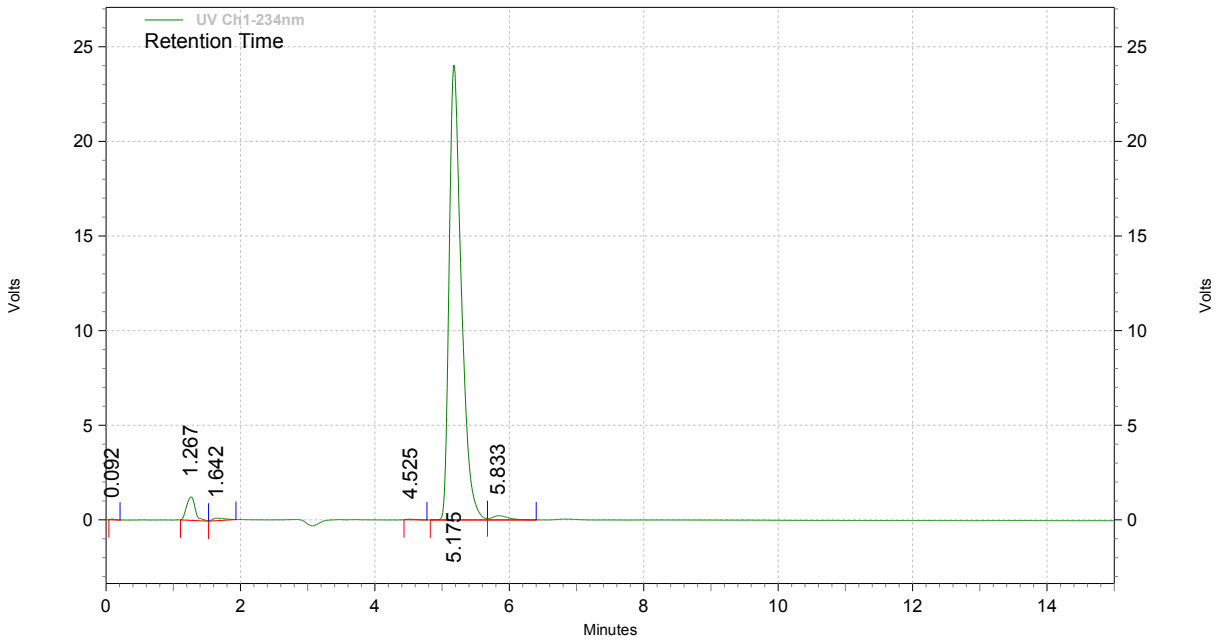
DEA 50 mg/L



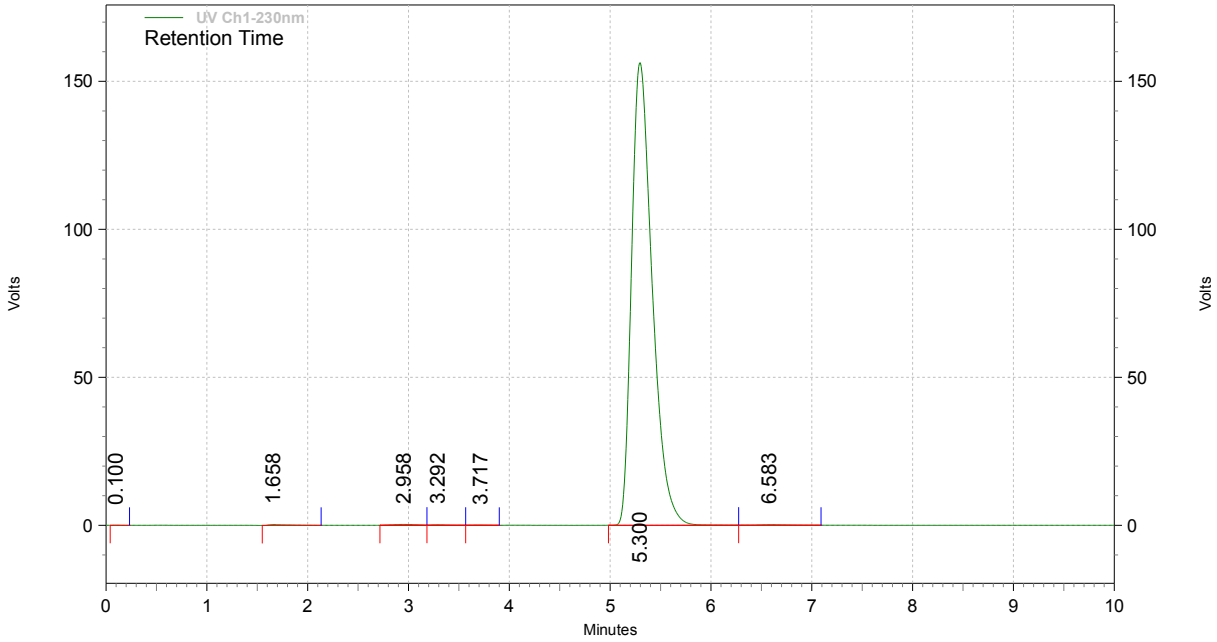
HA 1.56 mg/L



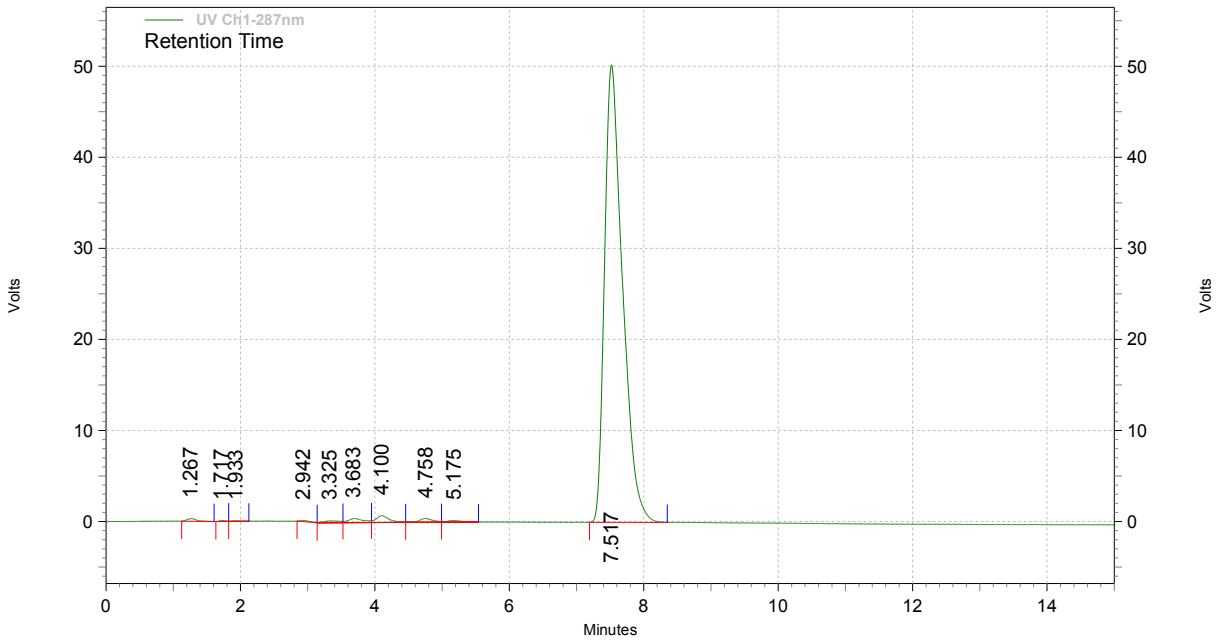
Cyanazine 8.63 mg/L



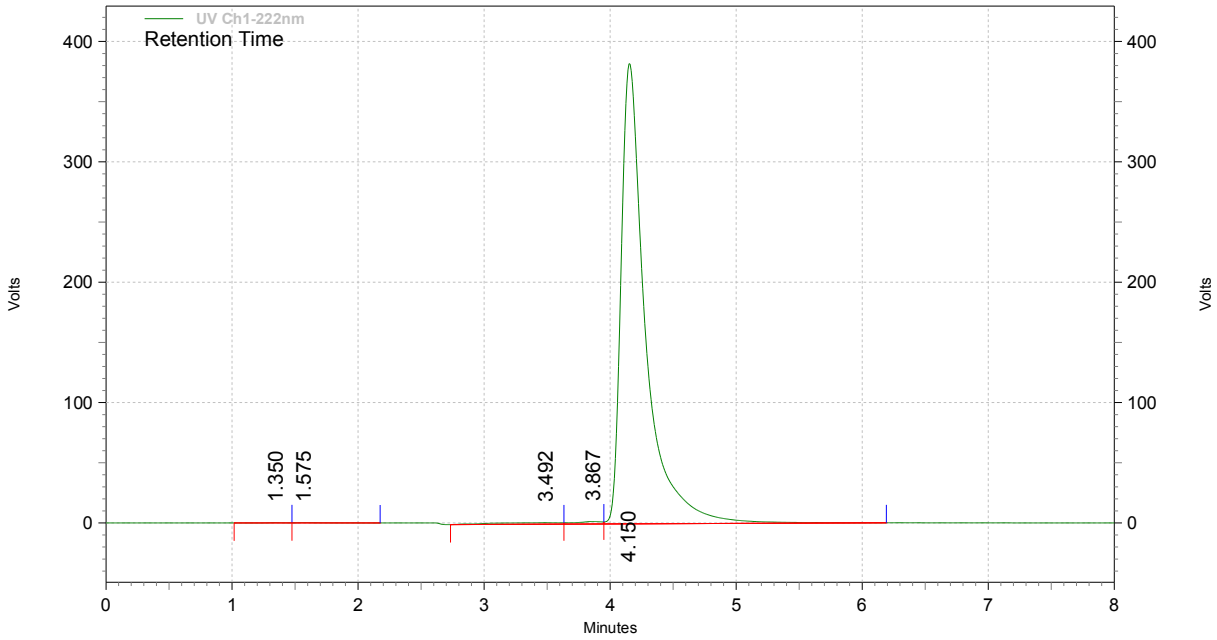
Simazine 31.25 mg/L



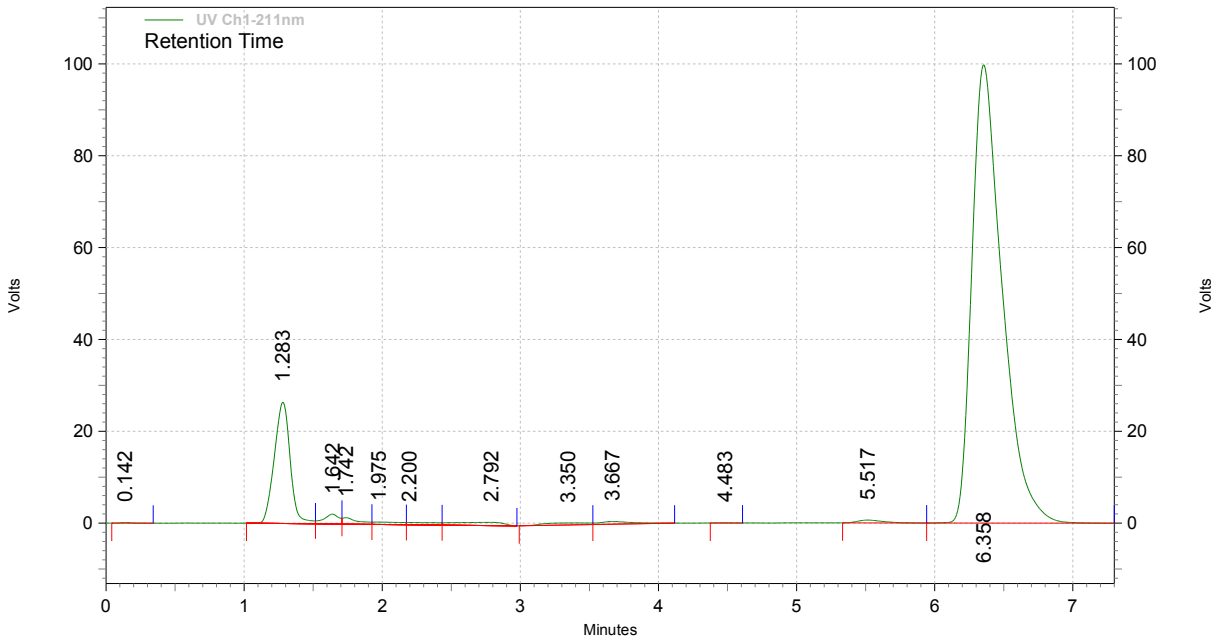
Nitrososimazine 50 mg/L



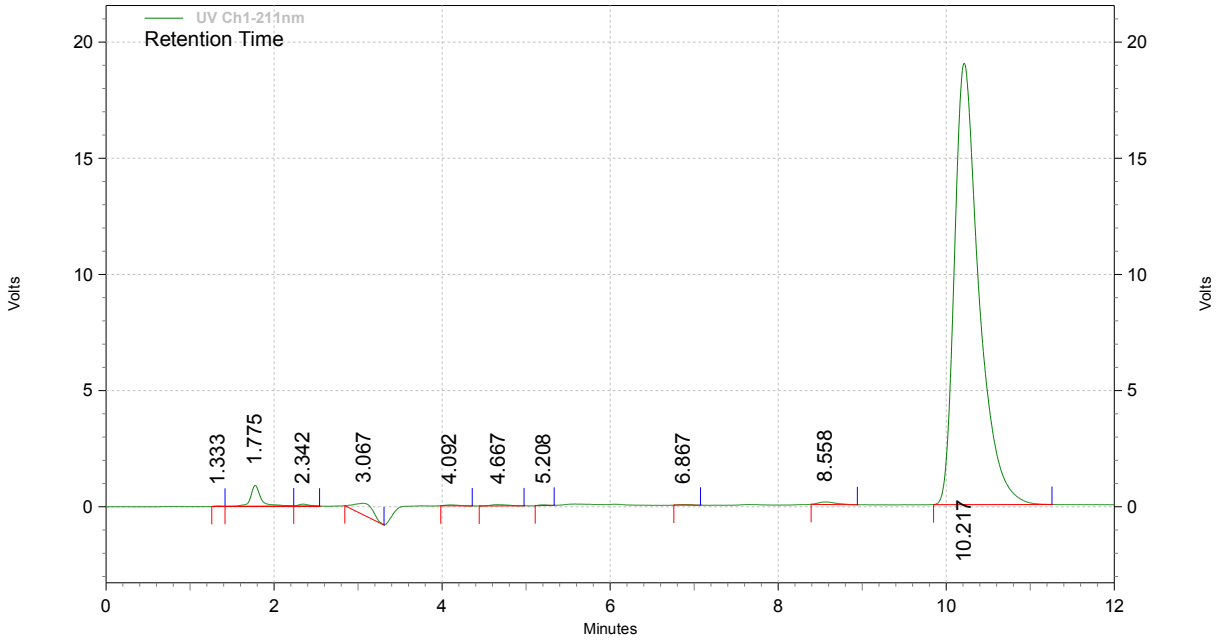
Ametryn 50 mg/L



Diuron 20 mg/L



Linuron 5.25 mg/L



Tebuthiuron 25 mg/L